Antenatal Exposure to Antibiotics and the Risk of Chronic Diseases in Childhood: A Population-based Study

By

Abin Chandrakumar

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University of Manitoba

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ABSTRACT

The maternal microbiome plays an important role in shaping the microorganism development in the infant's gut. The neonatal immune system development is driven by the gut microbiota and dysbiosis can precipitate chronic disease development during childhood. The aim of this study was to estimate the association of antenatal antibiotics and development of childhood chronic diseases study, in the 1996-2012 Manitoban birth cohort. Cox regression analyses were employed to determine these associations, and results are reported reporting them as adjusted hazard ratios (aHR) and confidence intervals (CI). Antenatal antibiotic exposure was significantly associated with an increased risk of severe allergy (aHR: 1.08; CI: 1.01-1.15), celiac disease (aHR: 1.59, CI: 1.46-1.71) and cholelithiasis (aHR: 1.46, CI: 1.21-1.77). Associations remained significant after being classified by antibiotic type. There was also a dose-response with increasing risk of celiac disease and cholelithiasis following multiple antenatal antibiotic exposures. The timing of antenatal exposure, however, did not modify the associations, and the associations for childhood celiac disease and cholelithiasis were similar for maternal antibiotic use during the nine months before and after pregnancy. Although our results revealed a potential risk of childhood severe allergy, celiac disease and cholelithiasis among offspring of mothers exposed to antenatal antibiotics, a similar risk associated with maternal antibiotic exposure during the 9-months before pregnancy and 9-months after pregnancy periods for the three diseases contradicts an exclusive association.

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ABBREVIATIONS

ABIS	All Babies in Southeast Sweden
aHR	Adjusted Hazards Ratio
ATC	Anatomical Therapeutic Chemical
B-cells	Beta Cells
CI	Confidence Interval
CIHR	Canadian Institutes Of Health Research
CMI	Canadian Microbiome Initiative
DC	Dendritic Cell
DER-CA	Diabetes Education Resource For Children And Adolescents
DPIN	Drug Product Identification Number
DOB	Date of Birth
Foxp3	Forkhead Box P3
GALT	Gut Associated Lymphoid Tissue
GIT	Gastrointestinal Tract
HFD	High Fat Diet
HLA	Human Leukocyte Antigen
HMO	Human Milk Oligosaccharide
HOSP	Hospital Discharge Abstract
HR	Hazard Rate
HMP	Human Microbiome Project
Ig	Immunoglobulin
IBD	Inflammatory Bowel Disease
ICD	International Statistical Classification of Diseases And Related Health Problems
IECs	Intestinal Epithelial Cells
IFH	International Scientific Forum on Home Hygiene
IFN	Interferon
IHMC	International Human Microbiome Consortium
IL	Interleukin
IR	Incidence Rate
LPS	Lipopolysacharide
MCHP	Manitoba Centre for Health Policy
MHSIP	Manitoba Health Services Insurance Plan
MHC	Major Histocompatibility Complex
NIH	National Institutes of Health
NOD	Non-obese Diabetic
NK	Natural Killer
OF	Old Friend
OR	Odds Ratio
PAMP	Pathogen-Associated Molecular Proteins
PCR	Polymerase Chain Reaction
PHIN	Personal Health Identification Numbers

PHYS	Physician Billing Claim
REGNO	Family Registration Number
SCFA	Short Chain Fatty Acid
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T_{effs}	Effector T-Cells
TGF	Tumor Growth Factor
Th	T-Helper
TLR	Toll-Like Receptor
T_{reg}	T-Regulatory
WHO	World Health Organization
WRHA	Winnipeg Regional Health Authority

Chapter 1. INTRODUCTION

Research on chronic diseases is mostly focussed on adults because the long-term morbidities are perceived as part of the ageing process. There has been a doubling in the prevalence of chronic childhood conditions during the past 50 years. Children often suffer from acute illnesses which are short-term and mostly infectious with some developing chronic conditions. There has been an epidemiological shift in the primary childhood disease burden from acute to chronic conditions [1]. Chronic disease is an umbrella term used for conditions which last more than three months whose sequelae affect a child's regular functioning, necessitating long-term hospital-based and home-based care for years or even lifelong. Van Cleave et al. defined chronic diseases in children as "any physical, emotional, or mental condition that prevented him or her from attending school regularly, doing regular school work, or doing usual childhood activities or that required frequent attention or treatment from a doctor or other health professional, regular use of any medication, or use of special equipment" [2].

The literature describes several reasons for the increase in the prevalence of childhood conditions. There could be an indirect result of dynamically changing definitions of chronic disease conditions and the enhanced disease diagnosis associated with improved healthcare access. Also, the advancement in therapeutics and antenatal care has increased the survival rates of children with morbidities which were lethal a few decades back. In North America, 98% of children with chronic diseases, which were previously fatal, survive to adulthood [3, 4]. Many children undergo remission from their disease after a few

years. However, chronic diseases in childhood predispose them to redevelop the condition again during adulthood, thereby having a prolonged implication on their well-being.

The prevalence of childhood chronic diseases has increased drastically in the past few decades from 12.8% in 1994, to 25.1% in 2000 and 26.6% in 2006 [5]. Extensive data available from different parts of the world suggest that environmental factors play as genetic factors. A study released by WHO in 2006 suggested that about 33% of the chronic diseases in children under five years are due to environmental exposures [6]. Approximately 14-18% youths in North America have a chronic disease, and a report released by the Canadian Council on Social Development estimated the number of children in Canada living with a chronic illness to be around half a million [7]. As per the 2011-12 Canadian data, 15.7% of children and youth aged 1-19 were living with asthma (IR: 1141.3 per 100 000), while 0.3% were living with diabetes (IR: 40.7 per 100 000) [8]. Chronic diseases whose incidence has increased could be classified as either allergic (food allergy [9], asthma [10, 11], atopic dermatitis [12] and rhinitis) or autoimmune (type 1 and type 2 diabetes, inflammatory bowel disease [13] and multiple sclerosis [14]). Reports have indicated that the incidence of both types of childhood diseases have doubled or tripled by the 1990s when compared to the 60s and 70s. Evidence from meta-analysis indicates that the data on increasing prevalence is real and not an outcome of changing diagnostic criteria. [15].

1.1. Cleanliness and Disease: The "Hygiene Hypothesis"

The anecdotal evidence that a decline in the prevalence of childhood acute infectious illness and a proportionate increase in hygiene/sanitation in the past century led to the postulate of the "hygiene hypothesis" by British epidemiologist Dr. David Strachan [16]. While following-up a cohort of 17,000 British children born in 1958, he observed that family size and birth order correlated with the risk of hay fever. In the paper, he remarked that having older siblings created a relatively unhygienic environment and likliehood of an early childhood infection which decreased the risk of eczema development in the younger siblings. This hypothesis came 20 years after Leibowitz et al. reported an augmented risk of multiple sclerosis among adults who spent their childhood in highly clean houses [17]. A study in Saskatchewan in 1976 had identified that the higher prevalence of helminth, viral and bacterial infections in the Metis people (residing in northern regions) was associated with relative freedom from asthma, urticaria and eczema in comparison to the relatively infection-free white community [18].

The term "hygiene hypothesis" however pre-dates the works of David Strachan and was initially used by David Barker to describe the increasing incidence of appendicitis [19, 20]. The scientific fraternity that considered infectious diseases during childhood as the trigger for allergy development initially viewed Strachan's hypothesis with skepticism. The hypothesis, however, gained momentum with the isolation of the T-helper 1 (Th1) and T-helper 2 (Th2) cell subsets in the 90s in animals as well as human models. The plasticity of the human immune system during infancy and exposure to infections was hypothesized to elicit a Th1 dominant response, thereby tilting the balance against Th2 dominance, which

leads to immunoglobulin E (IgE) mediated allergic phenotypes [21–23]. The hygiene hypothesis conceptualized that a child's immune system, like any other organ, requires "education" to mature and integrate into a fully functional system. The early exposure to infectious organisms and allergic triggers drives the "education" of our immune system in the right path and absence of such exposures could derail the maturation process, culminating in autoimmune and allergic manifestations. The hypothesis helped shift the then held paradigm of allergy development from an increased provocative agent exposure to the removal of a protective factor.

1.2. Epidemiological Support for the Hygiene Hypothesis

The epidemiologic support for the hypothesis came from numerous studies which found a positive association between lower socioeconomic status and lower frequency of immunologic diseases [24–26]. Although the postulate initially described the correlation of atopy and family size, the concept became broader encompassing autoimmune conditions as well. The notion was enshrined that an increase in hygiene because of westernization of societies was triggering the alarming increase in atopy and auto-immunity. While allergic conditions involve over-reaction of the immune system to potential triggers in the environment, autoimmune diseases are conditions where the immune system mounts an attack against own tissues (e.g. Type 1 diabetes, Crohn's disease, rheumatoid arthritis, celiac disease). A study in 1992 found that the incidence of type 1 diabetes mellitus (T1DM) in children born to Pakistanis who migrated to the UK was similar to the incidence of T1DM in native UK children. However, this rate was ten

times higher than the incidence observed in children born in Pakistan, where the socio-economic status is lower than the UK [27].

Interestingly, a study in the province of Manitoba by Blanchard et al. in 2001 resonated this association between hygiene and autoimmunity. The group observed a lower incidence of inflammatory bowel disease (IBD) in areas with lower average family income and higher average family size [28]. Even within countries like Ghana, the incidence of atopy was elevated in urban-rich children in comparison to the urban-poor and rural children [29]. In a landmark study published in 2002, Braun-Fahrländer et al. reported that the level of endotoxin (a cell wall component of gram-negative bacteria) in the mattress of children, which indicated the environmental microbial load, were lower in children with atopic conditions in comparison to non-atopic children [30]. They also observed that the exposure to farming in the initial years of life reduced the risk of atopy. Another interesting study from Detroit, a more urban environment, found that children who lived in houses having 2 or more pet dogs or cats may have reduced risk to multiple allergic sensitizations [31].

1.3. The Immune Mechanism of the Hygiene Hypothesis

Naïve CD4+T cells differentiate into Th1 and Th2 cells, which are usually balanced in a healthy individual. The autoimmune disease development is realized to be steered by IL-2 and IFN- γ which are secreted by Th1 cells. In contrast, cytokines IL-4 and IL-5 produced by Th2 cells are found to be linked with allergic diseases. An upregulation of Th1 cytokines is found to subdue the CD4+T cell maturation into Th2 cells and thereby allergic reactions. Pathogen-associated molecular proteins (PAMP) are components of

bacteria and virus that are recognized by Toll-like receptors (TLR) present on the natural killer (NK) cells and dendritic cells (DCs) leading to the production of several cytokines of which IL-12 stimulates the Th1 development [32]. The Th1 cells mount a cell-mediated response against the pathogens primarily via CD8+T cells and phagocytes. The absence of an early TLR stimulation by PAMPs leads to priming of Th2 cells that produce IL-4, IL-5, IL-9 and IL-13, which stimulates the IgE production by B-cells. The Th2 cells promote the maturation of eosinophil, basophils, mast cells, B-cells and suppress the phagocyte maturation, thereby promoting humoral mediated immunity. While the cytokine IL-4 evoked by Th2 cells inhibit the Th1 priming, IFN- γ produced by Th1 cell inhibits the Th2 cells. Until the end of the 20^{th} century, the immunological mechanism of the hygiene hypothesis was that at birth the immune system of neonates is Th2-dominant, and early life infectious exposures that prime the Th1 maturation helps attain a balance in the system [33, 34]. However, autoimmune diseases are characterized by cell-mediated destruction of the body and therefore a Th1 dominant pathway. Epidemiological data indicate that not just Th2 dominant allergies, but Th1 dominant autoimmune conditions are increasing in incidence among children [35]. A study published in Lancet by Steve & Nafstad in 2001 found a positive correlation between T1DM and atopy within and outside Europe, thereby refuting the implications of Th1/Th2 balance in disease development at the population level [36]. Also, the existing theory failed to explain the reason for reduced incidence of allergies in geographic areas characterized by chronic helminthic infections, which are considered to evoke Th2 dominance [37]. The inefficiency of deregulated Th1-Th2 balance to account for auto-immunity and allergy simultaneously led to the substitution of the general concept from missing immune deviation to decreased immune repression.

The T-regulatory (T_{reg}) cells, which have an immunosuppressive effect on Th1 and Th2 cells, was used as an alternative explanation during the 2000s. The development of Treg cells was considered dependent on continuous antigen exposure during childhood. [38–40]. There are several subsets of T_{reg} cells identified in the 1990s in animal models and subsequently in humans, which may be adaptive or innate in their action. The inducible/adaptive (iT_{reg}) type of cells such as T-regulatory 1 (Tr1) and T-helper 3 (Th3) are antigen-specific and act through secretion of IL-10 and TGF-β respectively. The innate CD4+ CD25+ regulatory T-cells (natural/nT_{reg}) do not require an antigenic stimulation and act through a contact-dependent mechanism without the involvement of cytokines. The identification of a transcription-factor Forkhead box P3 (Foxp3) in 2003, which controls the transition of naïve T cells to becoming the suppressor T-cells, was a landmark discovery in explaining the T_{reg} function [41, 42]. The cytokine TGF-β induces the expression of Foxp3 and deletion of the transcription factor leads to reduced suppressive activity by CD4+ CD25+ cells. Self-antigen reactive T-cells are present in both healthy individuals and autoimmune disease patients and are kept in check by the CD4+ CD25+ cells, and their poor or suppressed functioning leads to the development of autoimmune reactions [43–45]. Deletion of the suppressive activity of CD4+CD25+ cells by simultaneously administering high doses of IL-2 or anti-CD28 Ab before transfer to T-cell deficient mice was found to lead to autoimmune disease development [46]. In case of an infection, the cytokines drive the effector T-cells (T_{effs}), which are finally kept under control by the Treg cells once the pathogen load is brought down [47]. The increasing allergy epidemics could be explained by the double negative control on the Th2 skewing exerted by Th1 cytokines (IFNs and IL-

12) and the T_{reg} cytokines (TGF-β and IL-10) as a result of microbial exposure during the childhood.

1.4. Transition to the "Old Friend" (OF) Mechanism

The detrimental influence that the term "hygiene" in the hygiene hypothesis might have on the public perception of cleanliness, which could trigger infectious epidemics, prompted the International Scientific Forum on Home Hygiene (IFH) to recommend reviewing multiple aspects of the hypothesis. These included two fundamental issues: the clarification on the causative influence of cleanliness on the increasing atopy, and how clear was its impact on microbial exposure in comparison to other risk factors. The main report by Bloomfield et al. could not identify any significant causative links between the practice of hygiene and hygiene hypothesis [48].

It was observed in several epidemiological studies that infections during childhood do not protect from allergies, but potentiate them [49, 50]. In the 1980s it was observed that the germ-free rats had a moderately increased risk for development of adjuvant arthritis and the susceptibility could be altered by modifying the gut flora [51]. Similarly, mice which were kept in conventional facilities had a lower risk of arthritis in comparison to mice which were grown in isolated germ-free environments [52]. Studies in neonatal mice by Sudo et al. found that antibiotic administration which skewed the immunity to a Th2 predominance could be prevented by probiotic administration [53]. The role of commensal microbes in modifying the inflammatory response in ulcerative colitis was demonstrated

in mice models in multiple studies, indicating the role of non-pathogenic microbes in autoimmune disease progression [54, 55].

In 2003, Graham Rook proposed that it not be the infectious pathogens, but the non-pathogenic commensal microbes, microbes in our immediate surrounding and old pathogens in hunter-gatherer groups (helminths, salmonella, H.pylori, Hepatitis A, mycobacteria), that drive the development of the human immune system. He explained that the "crowd infections" could not persist in the small hunter-gatherer groups as it would either kill or impart immunity. From a Darwinian perspective, such infections were not a significant part of human evolution as humans started living as large populations only since approx. 10,000 BC. The human immune system started development much before that, and it could be the commensal microbes which co-evolved with humans that drive the immune system. However, selected fatal crowd infection epidemics might have had a role in eliminating specific HLA phenotypes (selective adaptation), but could never integrate into the immune development process as they eliminate the host. Rook indicated the absence of previously widespread pinworms in Europe around the 1960s and 70s coincided with the steady incline in the incidence of allergy. He described that the human immune system was developed in such a way that the immune response was not mounted against the non-pathogenic microbes and large-sized pathogens like helminths; the latter because an immune response might be insufficient to eliminate. This is evident from studies which found that the immune system was down-regulated in lymphatic filariasis to avoid unnecessary tissue damage [56]. The immune response against such organisms was dampened by an adaptive process of immunomodulation [57]. The substantiation of the link between the old friend (OF) microorganisms and chronic diseases comes from the

studies which showed that defective immunoregulation was evident in all these diseases and that certain micro-organisms in the gut induced remission in all these disease models through modulation of immunoregulation [58–60]. These OFs (*Lactobacillus reuteri* and *Lactobacillus casei*), when administered as probiotics, have demonstrated the ability to induce increased T_{reg} priming by DCs which partly promote the development of mucosal tolerance [61].

Modern lifestyle within hygienic concrete forests has resulted in reduced human contact with mud, animals and excreta, thereby losing contact with critical micro-organisms that guide influence the immunoregulatory circuits. In genetically susceptible individuals, the deficit of this critical restraint mechanism can manifest as allergies, autoimmunity or IBD. Rook also indicated that the exposure to OF microorganisms are most crucial during the initial years since birth based on the epidemiological findings that the children who were adopted to Sweden when below 2 years of age from low and middle-income countries had higher atopic prevalence in comparison to those who came between 2-6 years of age [62, 63]. The exposure during infancy, while the immune system is being established, with diverse non-pathogenic microorganisms primes the naïve system to tolerate a more extensive catalogue of organisms. The repertoire created as a result of the initial contact will not only include the organisms encountered but novel bacteria, archaea and viruses with similar molecular patterns.

1.5. The Human Microbiota

Super-organism was a term which originally indicated genetically similar organisms which function together as a single organism. However, recently the scope of the term was expanded to include diverse taxonomically distinct organisms functioning as a single unit. From the endosymbiotic mitochondria to the microbiota, humans have co-evolved in close connexion with cells from diverse genetic lineages, thus fitting the criteria for super-organism [64, 65].

The term “microbiota” is often interchangeably used with “microbiome” in medical-scientific literature. However, microbiota is the vast array of microbial cells harboured within and on the surface of the human body and microbiome refers to the genes contained in these cells. The aggregate count of microbiota was thought to outnumber human cells by 1:10 (10 trillion human cells to 100 trillion microbial cells) with a significant proportion of them inhabiting the gastrointestinal tract (GIT), especially the colon [66, 67]. This ratio was re-estimated as 1:3 by Bianconi et al., before being further corrected to 1:1 by Sender et al. [68, 69].

Similar to the genes that we have, which make us distinct from each other, the genomic composition of the microbiota (or microbiome) provides us with a unique microbiome signature which varies from person to person and also between various locations of the human body [70, 71]. Microbiota changes in regards to the niche location in the human body, by individual and also with time. Alpha-diversity is the term used to specify the species richness within a specific ecosystem (within-sample). Beta-diversity is

used to compare the species richness between two distinct (between-samples) ecosystems [72].

Since the exodus from East Africa, the environmental influences and changing lifestyle habits across the Neolithic to modern era has significantly altered the microbiome signatures. Even though microbiota comprises only 1-3% of our total mass, their role in metabolism, inflammation and immunity is considerable. The understanding that several chronic diseases in humans are manifestations of altered microbiota necessitated a deviation from traditional microbiology studies which focused the studies on individual species. The Human Microbiome Project (HMP) initiated by the National Institute of Health (NIH), USA and the Canadian Microbiome Initiative (CMI) initiated by the Canadian Institutes of Health Research (CIHR) seeks to characterize the human microbiome and explore the implications of microbiome alterations on human diseases. The vast amount of resources called for a collaborative approach that further led to the formation of International Human Microbiome Consortium (IHMC) in 2008, which coordinates the common goals of the microbiome initiatives around the globe [73, 74].

1.6. The Gut Microbiota

The gastrointestinal tract has an extensive surface area of approximately 250-400m^2 and provides a platform for interaction between host and antigens, given the fact that myriad pathogenic and non-pathogenic microbes pass through it along with what we take in orally each day [75]. The microbiota harboured in the intestine, termed as the "gut microbiota," is an essential part of the human body and is the most widely

studiedmicrobiota component due to its role in the development of metabolism and immune system. In contrast to the stomach and small intestine which harbour few species of microbiota, the large intestine, especially the colon is a densely populated diverse ecosystem [76, 77]. The composition of gut microbiota has evolved over thousands of years with human evolution to establish a symbiotic relationship. Its metabolic capacity transcends liver function by about 100 times and is therefore referred to as the obscure metabolic "organ" [78, 79]. Although harbouring a considerable number of archaea and eukaryotes (fungi and protozoa), bacteria dominate the gut microbiota composition. Despite the diversity, the phyla of bacteria that comprise the gut microbiota is only a tiny subset of the total bacterial phyla identified, indicating that extensive evolutionary selection was involved to establish a mutually beneficial gut microbiota [80]. Although initial culture-based studies claimed the species composition of gut microbiota across healthy individuals were more or less similar, recent studies under the HMP has found minimal phylogenetic overlap [81].

The gut microbiota comprises of 6 major phyla[1]: Firmicutes (*Ruminococcus*, *Clostridium*, *Lactobacillus*), Bacteroidetes (*Bacteroides*, *Prevotella*, *Xyalinobacter*), Proteobacteria (*Escherichia*, *Enterobacteriaceae*), Actinobacteria (*Bifidobacterium*), Fusobacteria and Verucomicrobia; among which the most dominant are Firmicutes and Bacteroidetes which comprise 90% of the total gut flora.

[1] Most important genera are provided inside parentheses.

1.7. Establishment of Human Gut Microbiota

Before the twentieth century, it was assumed that any microbial presence in the uterus would be detrimental to the fetal survival and that the infants are born sterile. Also, the intestinal microbes did not develop until the oral supplemental food was provided, as breastmilk was considered sterile as well. Henry Tissier, a French pediatrician, contradicted this statement by proposing that the neonatal gut is not sterile but contains microbes acquired the microbial inoculum while travelling through the birth canal [82]. However, he did not contradict the "sterile womb paradigm" and several studies which came in the following century found uterine infections to be associated with preterm delivery [83]. The dogma of the sterile womb came under scrutiny when it was found that cord blood amniotic fluid and fetal membrane contains PCR-detectable microbial genome in term healthy infants without conspicuous inflammation [84–87]. Jiménez et al. found that meconium, which is the first post-partum fecal excretion of ingested amniotic fluid and shed gut lining, contains a complex bacterial community. He fed mice with genetically labelled *E.faecium* inoculated milk and detected the presence of the inoculum in the meconium of their term offspring, corroborating his belief that the microbial transfer occurred from maternal GIT to the fetus[88]. However, the ethical concerns behind further studies have still kept us in darkness regarding the route through which the bacteria, archaea and viruses gain access to the uterine environment. The intestinal epithelial barrier prevents the translocation from the lumen into the bloodstream except if they are transferred by dendritic cells through luminal uptake [89, 90].

The external maternal transmission of microbiota occurs during the birthing process and is dependent upon the mode of delivery. It has been found that the microbiota of infants born through cesarean section has more resemblance to the skin microbiota in comparison to the vaginally delivered infants [91–95]. The maternal vaginal microbiota becomes less diverse with the progression of pregnancy and becomes enriched with lactobacilli, which is postulated to be crucial in establishing the post-partum upper gastrointestinal microbiota [96]. In contrast to the previously believed concept that breastmilk is sterile, microbiota has been isolated from the colostrum (first milk produced after delivery), and breast milk is found to significantly influence the early microbiota in infants. The microbial composition of breast milk which is initially abundant in lactobacilli changes by 6-months of lactation, probably to facilitate the switching of infants from liquid to solid food [97, 98].

Facultative anaerobes and aerobes initially colonize the infant gut which has positive redox potential at birth. The potential is lowered by these microbes that facilitate the colonization by obligate anaerobes which predominate the flora by the first week of life [99, 100]. Both formula-fed and breastfed groups have predominance of *bifidobacteria* in the intestine by about one month of age. Galacto-oligosaccharides in the breast milk promote the growth of *bifidobacterium* and therefore breastfed infants have higher *bifidobacteria* in comparison to formula-fed who have more diverse microbiota [101–103]. The *bifidobacteria*l count which is low during the first week starts to increase when breastfed in comparison to formula-fed infants. Initial microbiota is a result of vertical transmission while the flora is influenced by horizontal transmission during infancy as demonstrated by a study in pups. Only a few studies have identified that *Bifidobacteria*

strains from the vagina are detected in infant gut microbiota, paradoxical to the fact that vaginally delivered infants have fewer *Bifidobacteria* compared to that of microbiota of infants delivered through C-section [104, 105]. The microbiota becomes more complex by the end of the first year of life and becomes similar to that of adults by three years. The stability attained usually persists into adulthood unless acted upon by environmental insults.

1.8. Disruption of Gut Microbiota: Dysbiosis

Almost 2000 years back, Hippocrates stated that gut is the origin of all human diseases and the recently evolving understanding about the role of altered gut microbiota on non-communicable diseases substantiates this philosophy. Dysbiosis (a.k.a. dysbacteriosis) is the term used for definite change in the microbiota at any site in the human body which leads to the disruption of the homeostatic symbiotic relationship with the host. The term implies a disruption of the normal commensal microbiota in any niche in the human body and not just the gut, which in essence means a reduction in alpha-diversity (mean species diversity in a specific niche) [106]. Despite classifying humans into two types of enterotypes, there is substantial inter-individual variation in the gut microbiota at a species level, making the task of defining “normal microbiota” daunting. Dysbiosis can be either the loss of specific beneficial commensal flora or the overgrowth of certain resident flora elements that are detrimental. Pathobionts are species of organisms (e.g. Enterobacteriaceae) in the gut which are part of the normal flora which can induce pathology when their relative proportion in the niche expands [107, 108].

Dysbiosis usually leads to a decline in the overall species composition, especially reduction of obligate anaerobes and expansion of pathobionts and facultative anaerobes. The expansion of phylum Proteobacteria is considered the signature of dysbiosis and leads to reduced production of nutrients for the colonocytes which normally feed on the short chain fatty acids (SCFAs) produced by obligate anaerobes [109, 110].

1.9. The Functional Role of Human Gut Microbiota

The gut microbiome maintains a mutualistic relationship with the intestinal mucosa and contributes to the immune, metabolic and protective functions. The constituent microbiota, due to their diversity express enzymes is an efficient machinery to break down carbohydrates, lipids and proteins which serves as vital energy sources for the host. The microbiota in the colon, analogous to an anaerobic reactor, act on the dietary constituents which bypass the initial digestion process of the small intestine. The nutrients absorbed in the stomach and small intestine are products of host enzyme action. The remaining indigestible proteins and carbohydrates, which constitute 10-30% of the total energy consumed reaches the colon [111, 112]. Carbohydrate that reached the large intestine is acted upon by the obligate anaerobes and converted to simple sugars which are taken up by the enterocytes. Butyrate, a SCFA which is a product of carbohydrate and protein catabolism, is a major fuel for the colonocytes and enterocytes [76, 113]. These metabolic functions can either be depleted or escalate to noxious levels during dysbiosis. Many of these metabolites have local effect as well as effect on distant organs, which explains the wide spectrum of diseases that arise due to dysbiosis [114]. Vitamins which are readily

available from the ingested food are absorbed in the small intestine [115]. In addition to this, microbiota in the colon like *Bifidobacteria* and *Lactobacilli* process the ingested food to produce vitamin K and several water-soluble vitamins which are absorbed in the colon [116].

Based on a study in 2011 spanning several countries and races, humans can be classified into three enterotypes based on the species and functional composition of the gut ecosystem [117]. Despite harbouring abundant genus, the researchers identified that gut microbiota composition could be predominated by either *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) or *Ruminococcus* (enterotype 3). However, a study using a much larger population merged the *Ruminococcus* group, which was less well distinguished into enterotype 1 [118]. They found that the enterotype partitioning was independent of nationality, sex, age or body mass index, but strongly associated with long-term dietary habits. Components which predominantly come from animal sources such as protein and fat were found to promote the predominance of *Bacteroides* while plant sources promoted *Prevotella* dominance. The enterotype in which we fall not only decide how we process the nutrients but also determines the susceptibility to diet associated diseases. *Prevotella* enterotype are more effective at producing SCFAs which have a suppressive effect on inflammation and cancer [118, 119]. Bile acid metabolites have a carcinogenic effect and increasing *Bacteriodetes* promotes secondary bile acid production that leads to DNA damage, and precipitates IBD and cancer [120, 121].

A neonate's immune machinery has limited capacity to mount an innate immune response, and it is the pioneer flora that initiates the development of immune system. The evidence that gut microbiota play in the development of the immune system came from

studies in germ-free (GF) animal models and antibiotic-induced microbiota reconstitution studies [60, 122–125]. The models have demonstrated that exposure to gut microbiota in the post-natal setting drives the naïve neonatal immune system to develop tolerance to the new environment. Gross physiological changes such as defects in lymphoid organ development, distended cecum, decreased GI motility, abnormal enterocytes and smaller Peyer's patches have been observed in studies comparing the GF rodents with conventional rodents [126–128]. Though administration of commensals to GF mice could alter the condition, the correction tends to happen only if microbiota introduction is done at an early age. Extrapolating these findings to neonates implies that commensal establishment needs to happen within a specific age-window, in the absence of which there will be an irreversible loss of mucosal-immune homeostasis [129]. GF mice studies have established the importance of gut microbiota in the development of gut-associated lymphoid tissue (GALT) and developing the gut mucosal barrier [60, 130].

In addition to the physiological changes mentioned, GF mice were also found to have an impaired immune system which is Th2-skewed with a marked reduction in IgA, antimicrobial peptides and CD8+ cells [60]. The initial intestinal colonization drives the development of both innate and adaptive immune systems to safeguard against potential pathogens while simultaneously maintaining tolerance to non-pathogenic commensals. The LPS, which is an endotoxin on the outer membrane of gram-negative bacteria, is detected by toll-like receptors on intestinal epithelial cells (IECs), which prime the immune system to develop tolerance and enter into a state of hyporesponsiveness. This is achieved through IgA production by the GALT which coats the antigen and prevent further detection by the DCs. Some species of pioneer microbiota induce the production of TGF-beta which

further induces the production of Treg cells. Also, the SCFAs such as butyrate play an important role in immunomodulation by increasing the differentiation of naïve progenitor cells into Treg cells. The balance between Th1, Th2 and Th17 is essential to maintain a state of homeostasis which is facilitated by the Treg cells. The Th2 skewed immune system during birth is driven back to balance when the DCs sample *Bacteroides fragilis* from the lumen through induction of Th1 and T_{reg} responses by their polysaccharide A.

Similarly, segmented filamentous bacteria plays a major role in the activation of Th17 (the cytokine involved in resistance to autoimmune diseases and extracellular pathogens) through interaction with major histocompatibility complex II (MHC II) on enteric DCs [131]. Likewise, a wide range of commensals has been linked to the immune system maintenance. Despite all these findings, it remains unclear if species richness and diversity of microbiota is important or if it is the presence of certain species in combination that drive the development of the gut and immune system.

1.10. Post-Natal Factors Affecting Microbiota

The microbiota of the infant gut is in a dynamic state during the initial years of life that follows a population level pattern of species succession. Based on the timing of exposure, the factors that affect microbiota development could be stratified into the following:

The **mode of delivery** has been found to influence the pioneer microbiota established in infants. The microbiota abundance is lower and commensal diversity is found to develop at a much slower pace in C-section born infants in comparison to

vaginally delivered infants. The infants who are born via C-section delivery are colonized by environmental microbes (surgical equipment, other infants and healthcare practitioners) as opposed to the vaginally born infants whose microbiota has a greater resemblance to maternal vaginal microbiota [132]. The composition difference is evident at 3 months of age with *Bifidobacteria*, and *Bacteroides* being more prevalent in vaginal born infants against the predominance of clostridium and lactobacillus genera in C-section born infants. However, follow-up studies have indicated that the difference becomes less conspicuous from 6-12 months of age [133, 134]. Although using micro-birthing technique (topically applying maternal vaginal fluid on new-born children) to restore microbiota of C-section born infants was partially successful at restoring microbiota, the available evidence is incomplete [135]. A clinical trial (NCT02407184) that explored the benefits of vaginal delivery was prematurely withdrawn in January 2018 [136].

Feeding practices are not only a source of microbiota but an abundant supply of immunomodulatory molecules and bacterial nutrients. Breast milk contains hundreds of microorganism in addition to the human milk oligosaccharide (HMO) that acts as a prebiotic and influences the development of favourable microbiota in the neonate's gut [98, 137]. In comparison to formula-fed infants, breastfed infants are found to have a higher increase in the gut *bifidobacterium* and lactobacilli by 3 weeks of age [138, 139]. The alpha diversity of breastmilk is associated with maternal lifestyle, which leads to differences in microbiota seeding in the infant GIT. Recently, commercial milk formula has been altered to include different oligosaccharides that positively contribute to the bifidobacterial growth in the infant gut. However, the commensal composition in formula-fed infants still demonstrated an overrepresentation of *Bacteroides* and *Clostridium* [91, 140–142]. Mixed-

feeding is associated with a more diverse microbiota than exclusive breastfeeding. However, exclusive formula-feeding leads to reduced diversity [143].

Environmental influences in the post-natal period also play a strong influence in the microbiome development. The infants who are born into families in **rural locations** have exposure to more diverse environmental microbes and achieve a healthier microbiota in comparison to infants born in **affluent** urban families. Family members and siblings are major environmental influences with higher **birth order** found to decrease the risk of atopic conditions associated with dysbiosis. Antibiotics are one of the most commonly used classes of drugs in children, and several studies have found a positive correlation between **childhood antibiotic usage** and the development of chronic diseases during childhood [144, 145]. It is estimated that about 21% pediatric ambulatory visits and 30% of neonates are exposed to antibiotics, half of which are broad-spectrum [146, 147]. A Finnish cohort study found that the gut microbiota perturbation lasted for more than two years in children and macrolide class of antibiotics led to a relative increase in the Bacteroidetes phylum [148]. Numerous prospective and retrospective studies on childhood antibiotic use has found an increased risk of chronic disease development in exposed children versus non-exposed children [149, 150]. The risk was found to increase in a dose-dependent manner which indicates a possible causal relationship. Similarly, maternal antibiotic use in the peripartum period is also found to alter the microbiota in infants through the placenta as well as breastmilk [151, 152].

1.11. Maternal Influence on Infant Gut Microbiota

Genetics has been proposed to play a significant role in the initial microbiota composition based on the difference of microbiota between monozygotic twins, dizygotic twins and unrelated individuals. The compositional similarity was highest for monozygotic twins followed by dizygotic twins [153, 154]. Turnbaugh et al. contradicted this concept by proposing that genetically related individuals did have similar microbiota, but the difference between monozygotic and dizygotic twins was not significant [74, 155]. However, a recent study on healthy dichorionic triplet found that the monozygotic pair had more similarity in microbiota in comparison to the fraternal sibling, and the difference diminished by the age of 1 year [156]. Ever since the demonstration of bacterial genetic material in the placenta by Kovalovszki et al., which questioned the sterile womb paradigm, non-genetic maternal influences on the infant microbiota started gaining attention [157]. However, there are several factors which contradict the possibility of a maternal microbiota transfer. One is the impenetrability of the placental epithelium by commensals due to the presence of anatomical, physiological and immunological barriers. The sterile womb concept was formulated based on the findings that no viable bacteria were cultured from the placental components. Although PCR-based methods found bacterial DNA, no studies could isolate viable bacteria despite using culture methods that readily grow the same organisms in other body ecosystems. The microorganisms which were cultured from placenta and amniotic fluid after C-section had a composition more similar to skin microbiota indicating possible contamination. The presence of bacteria in meconium could also be the inoculum obtained in the post-natal setting from the mother, especially when there is significant time between delivery and meconium excretion. Lastly,

the procedures used for germ-free rodent production in itself points towards a possible sterile womb [158].

Although it is unclear as to the exact pregnancy trimester of the mechanism of intra-uterine translocation, the maternal microbiota is considered to exert a significant influence over the established pioneer microbiota. The maternal gut and vaginal microbiota signature is in a dynamic state during pregnancy progression, which might be a natural mechanism to facilitate the microbiota transfer [96, 159, 160].

The **duration of gestation** plays a major role in the type of pioneer microbiota being established. Establishment of a *bifidobacterium*-dominant gut is an important characteristic of healthy term-birth, which is hindered due to the overgrowth of enterococcus in the preterm gut [161]. **Obese and overweight** mothers have an altered microbiome with more *Bacteroides* (which inversely affect the growth of *bifidobacteria*) in comparison to healthy weight women. Even if delivered vaginally, the infants of such mothers tend to have a disrupted gut microbiome with reduced abundance of beneficial *bifidobacteria* [162, 163]. Moreover, the breast milk of obese mothers in contrast to normal-weight mothers have increased *Staphylococcus* and reduced *Bifidobacterium* [97]. Consumption of probiotics supplements during pregnancy has been found to reduce the incidence of allergy and also avoid preterm delivery. In comparison to other live probiotics which may promote bacteremia, administration of combinations containing *bifidobacterium* and *lactobacillus* 14 days before delivery was found to alter placental immune gene expression in pregnant women undergoing elective C-section [86, 164].

Termed the “restaurant hypothesis,” **maternal and infant diet** has an important role in shaping the gut ecosystem. Non-human primate studies have found that in comparison to

control diet, high-fat diet (HFD) during pregnancy period can lead to altered infant gut microbiome with reduced diversity and loss of key bacteria like Bacteroidetes [165].

Pregnancy is associated with hormonal, immune and metabolic changes, which are considered as an adaptive response to facilitate the fetoplacental unit growth and possible microbiota transfer. Hypothetically, any perturbation in maternal microbiota could influence the infant microbiota development as well. Antibiotics are among the most frequently used drugs during pregnancy, accounting for 80% of all prescribed medications. Among antibiotics, beta-lactams- especially penicillins, are the most commonly prescribed class due to their relative safety, followed by macrolides [166, 167]. Maternal antibiotic use could disrupt the hypothesized feto-placental microbiota exposure, change in the transplacental transmission of microbiota metabolites such as SCFAs, disrupt the configuration of the immune system through cytokine signalling and Ig associated transfer of commensal components, ultimately resulting in the development of an unstable fetal immune system [168]. Even if the sterile hypothesis does hold true, the dysbiosis induced by antibiotics could persist over several months which can lead to altered microbiota acquirement during delivery (vertical transfer) or during breastfeeding (horizontal transfer). Surprisingly, very few studies in humans have explored the alterations in the maternal and fetal microbiome or its long-lasting implications associated with antibiotic use during pregnancy. A clear causal link between maternal antibiotic use and the development of chronic diseases in their children has not been established in humans. Also, the role of maternal dysbiosis towards the development of disease phenotypes in the absence of genetic defects is still a major concern.

1.12. Dysbiosis, Antibiotics and Chronic Diseases

Genetic predisposition is vital, but not the sole contributory factor in the development of chronic diseases as has been shown in many sibling/twin studies. Given the rising incidence of childhood chronic diseases, and studies indicating the role of microbiota in the development and maturation of the immune system, exposures altering the microbiota in infants could play a significant role. The findings from previous studies that looked at the role of antibiotic use, dysbiosis and the development of chronic diseases are discussed below.

1.12.1. Childhood Severe Allergy

Although several studies have looked at the correlation between antenatal antibiotic exposure and atopy, no studies conducted so far have examined its association with severe allergic reactions. Moreover, when evaluating the impact of antenatal antibiotics on childhood allergy, asthma was combined with allergy to encompass all conditions falling within atopic/allergic march. The initial meta-analysis on the topic of childhood allergy (non-severe) was published in 2011 and included 3 studies evaluating the association between antenatal antibiotics and asthma. The results indicated a weak association and did not include a detailed discussion on confounders, subgroup analysis and literature quality [169]. Another meta-analysis published in 2015 combined the results from 10 publications evaluating the association between antenatal antibiotics and childhood risk for asthma/wheeze. The study found that antenatal antibiotic exposure increased the risk of the outcome in childhood, especially when the exposure was during the last 2 trimesters. However, there were only 2 studies that stratified the risk concerning trimester, and the overall effect size was biased by one study. However, the analysis did not account for confounding or information bias and recommended the need for larger cohort

studies [170]. Timm et al. found an association between maternal antibiotic use during pregnancy and development of atopy when antibiotics were taken during all three trimesters dermatitis in children born to atopic mothers only [171].

1.12.2. Celiac Disease

Celiac disease (gluten-sensitive enteropathy) is a disease characterized by an immune reaction to ingested dietary gluten. Long-lasting immune reactions cause a remodeling of the intestine leading to reduced nutrient absorption. A reduction in the abundance of Firmicutes and increase in Bacteroidetes, as well as Proteobacteria, has been observed in celiac disease patients irrespective of their age group. Although HLA-DQ2 and HLA-DQ8 have been implicated as the genetic risk factors for celiac disease development, only a minor proportion of these gene carriers develop the disease [172]. Germ-free mice with genetic susceptibility develop a much more severe gluten-induced disease phenotype in comparison to mice with normal microbiota, indicating the modulating influence that gut microbiota has on celiac disease [173]. A prospective questionnaire-based study published in 2014 did not find a statistically significant association between maternal antenatal antibiotics and development of celiac disease in children. However, the study had limited statistical power and did not account for the specifics of antibiotics used [174].

1.12.3. Type 1 Diabetes Mellitus (T1DM)

T1DM is a disease characterized by autoimmune destruction of pancreatic beta cells involved in the production of insulin, thereby resulting in insulin deficiency. The increase in pediatric T1DM and its correlation with evolving hygienic practices has led to the speculation

of a positive causal influence of microbiota on the development of T1DM. Both bio-breeding and NOD mice developed T1DM when they were kept germ free, indicating that microbiota might not have an independent causal influence on the disease development. However, the use of antibiotics which alters the gut microbiota has a modulating influence and a study in NOD mouse model found that antenatal maternal vancomycin led to accelerated T1DM development in offspring [175]. Another study was able to impart protection against T1DM in mice offspring by administering a cocktail of antibiotics to selectively eliminate gram-negative bacteria in the mother during the antenatal stage [176]. Comparison of fecal microbiota found an increased abundance of *Bacteroides* and decreased *bifidobacterium* in children with β-cell autoimmunity in comparison to those without autoimmunity [177]. Despite these findings, it is unclear as to the mechanism of how the disruption of microbiota results in an organ-specific dysfunction. A possible mechanism has been the alteration in butyrate-induced maintenance of gut permeability and an alteration induced by the declining butyrate-producing bacteria because of antibiotic usage. A Finnish population based cohort study did not find a significant association between maternal antibiotics before pregnancy, during pregnancy or childhood antibiotics and T1DM development [178]. However, they did find a dose dependent relation and cumulative effect when both mother and child used antibiotics. Also, certain antibiotics such as quinolones and phenoxymethylpenicillins before pregnancy was found to be associated with an increased risk of T1DM development. Although the authors felt that the evidences were inconclusive, they speculated the possibility of persistence of quinolone in the mothers through deposition in bones, which might have influenced the infant microbiome.

1.12.4. Type 2 Diabetes Mellitus

T2DM is a disease characterized by a state of insulin resistance accompanied by low-grade inflammation. Larsen et al., through real-time quantitative PCR analysis of fecal microbiota, found that the gut microbiota composition of diabetic individuals is different from that of non-diabetics [179]. Also, they found evidence that an elevated Firmicutes/Bacteroidetes ratio and reduction in *bifidobacterium* was associated with the development of T2DM. Lipopolysaccharide (LPS), which is a component of the gram-negative bacterial cell wall, increases in subjects with high fat intake and is associated with induction of low-grade inflammation. LPS circulates in the plasma in bound form to chylomicrons after being absorbed by the enterocytes, binds to CD14/TLR4 receptors on the macrophages and induces production of pro-inflammatory cytokines. A change in the number and composition of gram-negative bacteria as a result of antibiotic exposure can, therefore, lead to increased LPS, which in a genetically pre-disposed individual could make them susceptible to diabetes development [180]. Studies in both humans and mice have found that childhood use of broad-spectrum antibiotic leads to obesity, which shares etiology as well as is a major risk factor for T2DM [181]. Despite the clarity that antibiotics alter gut microbiota, there have also been few conflicting studies showing an increased insulin sensitivity and reduced body weight [182, 183]. Another study from 2012 showed that the fecal transplantation from lean donor to individuals having obesity or metabolic syndrome caused an improvement in insulin resistance [184].

1.12.5. Cholelithiasis

The condition, also known as gallstones, is a chronic hepatobiliary condition which involves deposition of gallstones in the gallbladder, hepatobiliary tract or common bile duct as a result of impaired metabolism of bilirubin, bile acids and cholesterol [185]. Despite significantly influencing gallstone formation, genetic factors have been identified to contribute only 25% of the overall phenotypic variation among twins [186]. Although bile was considered sterile before, and bacterial presence in the bile is indicative of hepatic morbidity, high throughput sequencing studies have found the presence of microbiota in the biliary tract. Further evidence also indicated that the perturbation in the intestinal microbiota had a causative influence on the development of several hepatobiliary diseases through regulation of bile acid metabolism [187]. About $3/4^{th}$ of patients with the fibroinflammatory condition primary sclerosing cholangitis was found to have inflammatory bowel disease, whose underlying etiology involves perturbation in the gut microbiome [188]. The persistent healthy interaction of the gut microbiota with the liver (gut-liver axis) is essential in maintaining metabolic homeostasis, and an altered microbiota is believed to play a significant role in the development and progression of hepatobiliary diseases. Although the incidence of pediatric gallstones is lower than in adults, the numbers are on the rise, which might be a result of better diagnostic facilities available or due to increased obesity in teens. This change has also been a result of the shift in etiology of pediatric cholelithiasis from hemolytic diseases to biliary dyskinesia and non-hemolytic pediatric diseases [189].

1.13. The rationale for the Study:

An increasing amount of data indicates that the infant's initial microbiome has a maternal signature and the mother's intestinal microbiota contribute to the fetal and infant immune system development. Although not all human studies found an association, the majority of the published literature was supportive of the hypothesis that antibiotic consumption by mothers during pregnancy increases the risk of atopy development in their offspring. Broad spectrum antibiotic consumption leaves a long-lasting impact on the maternal gut microbiota which can be transmitted to infants through vertical or horizontal transfer.

One of the major drawbacks in the methodology in the available epidemiologic studies was described by Stokholm et al., who did a sensitivity analysis by comparing the risk of childhood asthma based on maternal antibiotic use 80-weeks before pregnancy, during pregnancy and 80-weeks after parturition [190]. They hypothesized that if the antibiotic use were a surrogate marker of the increased maternal propensity for infection and maternal lifestyle, the association would not be different around a window of 200 weeks surrounding pregnancy. The association was found to be present even with maternal antibiotics 1.5 years after pregnancy, which is presumed to have minimal influence on the vertical transmission of gut microbiota. The lack of a temporal relationship contradicted the possibility of antibiotic use or inflammatory processes causing an intrauterine outcome. A commentary on the paper indicated that the cumulative exposure to antibiotics in the gestational and pre-gestational period could be transmitted to the infants during pregnancy due to long-lasting dysbiosis [191]. Also, post-natal antibiotics in mothers might lead to the transfer of perturbed flora during breastfeeding or microbiota transferred through pre-mastication of food by mothers. Increased antibiotic use

among mothers could be an indication of maternal or physician inclination to use antibiotics copiously, and this would reflect in their infant's medication use as well. Highly lipophilic drugs such as quinolones and macrolides can accumulate in tissues at a relatively higher concentration than in plasma and use of such antibiotics during pregnancies or even pre-pregnancy period could create repositories which have a long-lasting impact on maternal and infant microbiota [178].

Previous studies evaluating the impact of early-life antibiotic exposure have focused mainly on asthma, atopy, and allergy but no single study has evaluated and compared more chronic diseases in that respect. Also, very few studies have determined associations by type of antibiotic, the number of courses prescribed, trimester of exposure and maternal propensity for infection. Accordingly, we conducted this population-based study to examine the association between maternal antibiotic exposure during pregnancy and development of five chronic diseases in their offspring using administrative health data comprising of Manitoban children from the year 1996 to 2012. Furthermore, we categorized the antibiotic exposure for the type of antibiotic, number of antibiotic prescriptions, trimester of antibiotic use and adjusted for several *a priori* covariates.

Chapter 2. METHODOLOGY

Using health administrative data of people in Manitoba, Canada, housed at the Manitoba Centre for Health Policy (MCHP), we undertook a retrospective cohort study of mother-infant dyads in Manitoba, Canada. Our provincial birth cohort included all children who were born in Manitoba between 1996 and 2012. We used the following databases: physician billing claims, drug program information network (DPIN), hospitalization discharge abstracts, Canada Census data, the Diabetes Education Resource for Children and Adolescents (DER-CA) database and the Manitoba Health Services Insurance Plan (MHSIP) as reliable and valid data sources. The methodology used for our study was an extension of the methods used by Loewen et al. in the study evaluating the association of maternal antibiotic use during pregnancy period and childhood asthma [192].

1) **Medical/Physician Claims:** Also known by the name physician billing claims, this information is contained in the Medical Services Database and contains the claims submitted by physicians to the provincial government for service provided. It comprises of information such as the patient who sought the service, provider involved, the specifics of service provided including related fee. For physicians who are on an alternate payment plan (session rate, contracts etc.), there is a system of "shadow billing" submitted by physicians .

2) **Drug Program Information Network (DPIN):** The database connects all pharmacies in Manitoba with Manitoba Health and is updated on a continuous basis. Regardless of the type of coverage, this database captures information regarding prescriptions and

dispensed medications at the point-of-sale. However, the data does not include hospital dispensation and the over-the-counter drug data recorded depends on the formulary used,.

3) **Hospitalization Discharge Abstracts:** This is a subset of the hospital abstracts and comprises demographic, administrative and clinical summaries submitted by the hospitals upon the discharge of the patients from acute or chronic hospitalization to Manitoba Health. This includes the abstracts of residents and non-residents of Manitoba admitted in any provincial healthcare facility as well as the abstracts of Manitobans admitted in hospitals outside the province. The disease information is recorded as per the International Statistical Classification of Diseases and Related Health Problems (ICD 9-CM till March 31, 2004, and ICD 10 from April 1, 2004).

4) **Diabetes Education Resource for Children and Adolescents (DER-CA) database:** The databank contains information on children with T1DM and T2DM in Manitoba since January 1986. The child health data comprises individual demographic, clinical and laboratory records sourced from the Winnipeg Regional Health Authority.

5) **Manitoba Health Services Insurance Plan (MHSIP):** The population-based archive, instituted and maintained by Manitoba Health since the 1970s, comprising of individual data on demography, family, residential location, date of birth (or immigration to Manitoba) and death (or emigration from Manitoba). The concise form of data is transferred by Manitoba Health to MCHP on a semi-annual basis. In this study, we used the drug prescriptions collected by the MHSIP to obtain the data on antibiotic usage.

Within the family, each individual is assigned a 6-digit personal health identification number

The linkages among the mother and their offspring was obtained using family registration number (REGNO). De-identification of the data is obtained by removing any patient-specific details in the MCHP database that prevents the potential identification of the patient using the data. Encrypted personal health identification numbers (PHIN) are provided to MCHP by Manitoba Health and helps to find temporal individual-level associations. In the absence of a PHIN, REGNOs-SEX-DOB-INITIALS are used by MCHP to create alternate PHINs which are distinct from the PHIN assigned by Manitoba Health. Starting from 1966, REGNOs were assigned to Manitoba residents and comprises 9-digits which helps to identify family units that receive care.

The scrambled PHIN and REGNOs were used to link the healthcare data of the mother with that of offspring. Only those mothers who were registered with the MHSIP one year before and one year after pregnancy were included. This was to ensure the capture of maternal antibiotic exposure in the pre-pregnancy and postnatal settings in addition to antenatal exposure. For the mother-infant dyad to be included, the children born to mothers meeting the criteria had to be continuously registered with the MHSIP for 3-year postpartum. The study received ethical clearance form both the Health Research Ethics Board at the University of Manitoba and the Health Information Privacy Committee.

2.1. Primary Exposure of Interest: Antenatal Exposure to Antibiotics

The systemic antibiotic usage in maternal prescription records was used as an indicator of antenatal exposure. The antibiotic usage was classified based on:

- Frequency: the number of antibiotic courses prescribed.
- Timing: trimester of exposure, estimated using the birth date of the child while considering the documented gestational age in infant records. Other than the timing of exposure during pregnancy period, the antibiotic exposure 9-months before pregnancy and 9-months after parturition were assessed. Also, early childhood antibiotic use up to three years from birth was assessed and stratified as 0-3 months, 3-6 months, 6-12 months and beyond 12 months.
- Type of antibiotic: based on WHO Collaborating Centre for Drug Statistics Methodology, stratified into (five groups): Beta-lactam penicillins (J01C); other Beta-lactams (J01D); Macrolides, streptogramins, lincosamides (J01F); Tetracyclines, quinolones, others (J01A, J01M, J01X); Sulphonamides and trimethoprim (J01E).

2.2. The outcome of Interest: Childhood Chronic Diseases

Chronic disease outcome in children was evaluated using the MCHP healthcare administrative data. Specific ICD codes (ICD-9 and ICD-10) and Anatomical Therapeutic Chemical Classification System (ATC) codes were used as indicators of outcome.

1) Severe Allergy: defined as receiving a minimum of 2 prescriptions of intramuscular epinephrine (where each prescription is at least 180 days apart) OR receiving a minimum of 1 hospital diagnosis (ICD codes for the condition is described in Table 1). Either of

these needs to be observed on or after an individual's 3rd birthday to be counted. For those individuals that met these criteria after their third birthday, we then looked at the earliest date either of these two criteria was met and used that as the date of onset.

2) Celiac Disease: Any hospitalization or ≥1 physician diagnosis identified using the ICD codes of celiac disease specified in table 1.
3) Maternal Diabetes: Maternal diabetes was described as any hospitalization OR presence of ≥ two physician diagnosis for diabetes using the ICD codes of diabetes specified in table 1 OR receiving a minimum of 2 prescriptions of drugs in ATC class A10 (drugs used in diabetes).
4) Type 1 Diabetes Mellitus (T1DM): Patient in DER-CA database
5) Type 2 Diabetes Mellitus (T2DM): Patient in DER-CA database
6) Cholelithiasis: Any hospitalization or ≥1 physician diagnosis identified using the ICD codes of cholelithiasis specified in table 1.

We used specific International Classification of Diseases (ICD) codes (ICD-9 and ICD-10) to create these indicators (Table 1).

Table 1: ICD codes used to identify the disease outcome in children [ICD-9-CM (before 2004), ICD-10-CA (post-2004)]

Condition	Data Source	Coding System	Code Matching Pattern
Severe Allergy	DPIN	ATC	C01CA24
	HOSP	ICD-9	995.0 *
	HOSP	ICD-9	995.1*
	HOSP	ICD-9	995.2*
	HOSP	ICD-9	995.3*
	HOSP	ICD-9	995.4*
	HOSP	ICD-9	995.6*
	HOSP	ICD-10	T78.0*
	HOSP	ICD-10	T78.1*
	HOSP	ICD-10	T78.2*
	HOSP	ICD-10	T78.3*
Cholelithiasis	PHYS	ICD-9	574
	HOSP	ICD-9	574.*
	HOSP	ICD-10	K80.*
Celiac Disease	PHYS	ICD-9	579
	HOSP	ICD-9	579.0*
	HOSP	ICD-10	K90.0*
Diabetes	PHYS	ICD-9	250
	HOSP	ICD-9	250.*
		ICD-10	E10.*
			E11.*
			E12.*
			E13.*
			E14.*
			O24.*
			G59.0*
			G63.2*
			H28.0*
			H36.0*
			M14.2*
			M14.6*
			N08.3*
	DPIN	ATC	A10.*

PHY: physician billing claims, HOSP: hospital discharge abstract, ATC: Anatomical therapeutic chemical classification, ICD: International Statistical Classification of Diseases and Related Health Problems
*wildcard used

Potential confounders

Details of several variables were extracted from the databases.

Demographic covariates

- Infant Sex
- Gestational age
- Birth weight
- Birth order
- Mode of birth (caesarean or vaginal delivery)
- Multiple births (yes or no)
- Breastfeeding initiation (exclusive, partial, formula-fed)
- Year of birth
- Season of birth: classified as Winter, Spring, Summer, Fall
- Rural/urban living
- Socioeconomic status: Q1 is the lowest income quintile, and Q5 is the highest [193]
- Number of children in the household (may or may not be siblings)
- Number of siblings: maternal parity was used to calculate the number of siblings at birth
- Child age at diagnosis
- Maternal age

Health-related covariates

- Maternal history of chronic disease in question
- Antibiotic prescription in the first year of life
- Maternal hospital utilization

- Maternal frequency of physician visits
- Child healthcare utilization: number of healthcare visits (physician + hospital) during the first year of life

2.3. Statistical analysis

The descriptive representation of both maternal and child demographic characteristics was done using frequency table generation for categorical variables. Continuous variables such as maternal age were represented as a mean ± standard deviation, and duration of follow-up was represented as the median value. Survival analysis was used to measure time from the child's birthdate to the initial occurrence any of the following events:

- The earliest date on which the data of the child matched the disease definition (mentioned previously).
- Death of the child
- The child was lost to follow-up
- Loss of coverage (which is a result of individual emigration from the province)
- The date of study conclusion.

The survival distribution of our sample was described using survival functions (hazard ratio, survivor fraction) and was further used for univariate, bivariate and multivariate analyses. Non-parametric (life table, Kaplan-Meier) and semi-parametric survival (Cox regression) analysis was done by considering the timing of change as a continuous variable. We converted the person-data to person-time data to examine associations between antibiotic exposure and development of childhood chronic diseases. A Cox regression model was used as a distribution-free model to

report crude (HR) and adjusted hazards ratios (aHR) with their 95% confidence intervals. Confounders which were considered a priori (infant sex, antibiotic use in infancy, socioeconomic status, and location of residence) or those risk factors which significantly affected the crude hazards ratio (positive maternal history of the disease under consideration, gender, length of gestation, number of children in household) when adjusted were used in the final model to estimate the risk associated with antenatal antibiotic exposure. The dose-effect relationship was tested by modelling antibiotic exposure as a continuous or ordinal variable and examining the pre-pregnancy, pregnancy and post-pregnancy antibiotic exposure.

Furthermore, we conducted a sensitivity analysis by comparing the aHR of antenatal exposure to the aHR of maternal antibiotic exposure in the pre-pregnancy and post-pregnancy setting. The exposure during the pre-pregnancy and post-pregnancy exposure window was estimated with reference to offspring's date of birth and gestational age. Modelling of interaction elements was performed to identify any modification in the final effect by any of the covariates, and maximum likelihood ratio test was used to test for the significance of interaction terms.

The assessment of an acceptable degree of Cox-regression model fit for the data was done through a model run and Chi-square test by performing a likelihood ratio test to compare -2 log likelihood between a model with all covariates and a null model (with no covariates). This test is subject to a chi-square distribution with a given degree of freedom including covariates. A p-value <0.05 indicated a good model fit. To run Cox regression analysis, we first checked the two following assumptions:

1. Non-informative censoring (where drop outs/censoring happen due to reasons which are not related to the outcome under consideration): To satisfy this assumption, the current study

design ensured that the mechanisms giving rise to the censoring of participants are not related to the probability of chronic disease development.

2. Proportional hazard assumption (the hazard functions of the survival curves for the exposed and non-exposed groups are proportional concerning time): To check this assumption, we ran log-log survivor plot.

We performed all data management, programming, and analyses using SAS® statistical analysis software, version 9.4 (SAS Institute Inc., 2011). For between-group comparisons, we considered a p-value≤0.05 as statistically significant.

2.4. Subgroup/ Sensitivity Analysis

We performed subgroup and sensitivity analyses on the primary outcome classified by the following variables:

1. Types and classes of antibiotics

1.1. Groups: ATC codes J01A to J01X

2. Timing of exposure

2.1. Grouped as antenatal exposure during first second or third trimester

3. Type of exposure

3.1. Both indirect exposure through maternal antibiotic utilization and direct exposure through post-natal childhood antibiotic use

4. Frequency of exposure

4.1. Data were categorized as no exposure, 1 course of antibiotic, 2 courses of antibiotic or ≥3 courses of antibiotics

5. The maternal propensity for infection by examining her use of antibiotics 9-months before and 9-months after pregnancy.

5.1.Antenatal exposure: antibiotics dispensed to a mother during pregnancy

5.2. Pre-pregnancy: antibiotics dispensed to mother nine months before pregnancy

5.3.Post-pregnancy: antibiotics dispensed to mother nine months after pregnancy

Chapter 3. RESULTS

3.1. Demographics of Maternal Antibiotic Exposure during Pregnancy

There were 235,891 live births in the province of Manitoba between 1996 and 2012. We successfully obtained a linkage between 213,661 mother-infant dyads, with the study population having a median duration of 10.5 years of follow-up from birth. The mean age of mothers, who were part of our study population, at parturition was 27.64±5.88 years. Overall, there were 78,522 (36.8%) mother-infant dyads who were exposed to antibiotics during the pregnancy period. The characteristics of antibiotic exposure in the total population, irrespective of the outcome are provided in Table 2. A slightly higher proportion of women from the rural neighbourhood (39%) had antibiotic exposure during pregnancy than women from an urban neighbourhood (34.8%). Among others from families with the lowest income quintile (Q1) 41.8% had antenatal exposure, while only 31.6% mothers from the most affluent families (Q5) received antenatal antibiotics. Women who delivered preterm infants had a marginally higher antibiotic exposure during pregnancy than women are delivering at term. Among the 41,248 women who underwent a cesarean section, 37.7% were exposed to antibiotics during pregnancy. The proportion of antenatal antibiotic exposure was similar in multiple births and singleton pregnancies (36.7% vs 37.9%). A lower proportion of *Primagravida* (parity=0) had antibiotic exposure during pregnancy in comparison to primiparous (parity=1) and multiparous (parity>1) women. Pregnant women living in a household with 4 or more children had a higher proportion of antibiotic use (39.9%) that those living in a household with one child (34.7%). Antibiotic exposure during pregnancy was more in women who had higher physician utilization. Physician utilization of >29 instances accounted for 29.8% of the total antibiotic exposed group.

Table 2: Characteristics of maternal antenatal antibiotic exposure

Variables		***Antenatal Exposure***			
		No (n=135,139)		***Yes (n=78,522)***	
		Frequency (%)*	***% $***	***Frequency (%)****	***% $***
Antenatal Exposure					
	No	*135,139 (63.2%)*	*N/A*	*N/A*	*N/A*
	Yes	*N/A*	*N/A*	*78,522 (36.8%)*	*N/A*
No. of Antenatal Courses					
	0	*135,139 (63.2%)*	*N/A*	*N/A*	*N/A*
	1	*N/A*	*N/A*	*47,286 (22.1%)*	*N/A*
	2	*N/A*	*N/A*	*17,954 (8.4%)*	*N/A*
	≥3	*N/A*	*N/A*	*13,282 (6.2%)*	*N/A*
Type of Antibiotics					
	J01C	*N/A*	*N/A*	*52,598 (24.6%)*	*N/A*
	J01D	*N/A*	*N/A*	*12,998 (6.1%)*	*N/A*
	J01F	*N/A*	*N/A*	*15,120 (7.1%)*	*N/A*
	J01(A, M, X)	*N/A*	*N/A*	*16,074 (7.5%)*	*N/A*
	J01E	*N/A*	*N/A*	*5,615 (2.6%)*	*N/A*
Trimester of Exposure					
	Trimester 1	*N/A*	*N/A*	*34,562 (16.2%)*	*N/A*
	Trimester 2	*N/A*	*N/A*	*39,340 (18.4%)*	*N/A*
	Trimester 3	*N/A*	*N/A*	*31,330 (14.7%)*	*N/A*
Gender					
	Male	*69,170 (51.2%)*	*63.2%*	*40,267 (51.3%)*	*36.8%*
	Female	*65,969 (48.8%)*	*63.3%*	*38,255 (48.7%)*	*36.7%*
Residence Locality					
	Urban	*75,358 (55.8%)*	*65.2%*	*40,246 (51.3%)*	*34.8%*
	Rural	*59,493 (44.0%)*	*61.0%*	*38,056 (48.5%)*	*39.0%*
	Unknown	*288 (0.2%)*	*56.7%*	*220 (0.3%)*	*43.3%*
Income Quintile					
	Q1 (lowest)	*32,593 (24.1%)*	*58.2%*	*23,426 (29.8%)*	*41.8%*
	Q2	*27,577 (20.4%)*	*62.4%*	*16,637 (21.2%)*	*37.6%*
	Q3	*25,298 (18.7%)*	*63.6%*	*14,499 (18.5%)*	*36.4%*

	Q4	*26,055 (19.3%)*	*66.8%*	*12,962 (16.5%)*	*33.2%*
	Q5 (highest)	*23,328 (17.3%)*	*68.4%*	*10,778 (13.7%)*	*31.6%*
	Unknown	*288 (0.2%)*	*56.7%*	*220 (0.3%)*	*43.3%*
Gestation Period (wk.)					
	<35	*3,054 (2.3%)*	*60.9%*	*1,962 (2.5%)*	*39.1%*
	35 to <37	*5,598 (4.1%)*	*60.9%*	*3,594 (4.6%)*	*39.1%*
	37 to <39	*27,370 (20.3%)*	*62.5%*	*16,429 (20.9%)*	*37.5%*
	≥39	*99,117 (73.3%)*	*63.7%*	*56,537 (72.0%)*	*36.3%*
Birth Weight (gm)					
	<3000	*23,193 (17.2%)*	*63.0%*	*13,640 (17.4%)*	*37.0%*
	3000 to <3500	*45,166 (33.4%)*	*64.0%*	*25,353 (32.3%)*	*36.0%*
	3500 to <4500	*62,523 (46.3%)*	*63.1%*	*36,626 (46.6%)*	*36.9%*
	≥4500	*3,958 (2.9%)*	*60.2%*	*2,613 (3.3%)*	*39.8%*
	Unknown	*299 (0.2%)*	*50.8%*	*290 (0.4%)*	*49.2%*
Delivery Method					
	C-Section	*25,691 (19.0%)*	*62.3%*	*15,557 (19.8%)*	*37.7%*
	Vaginal	*109,448 (81.0%)*	*63.5%*	*62,965 (80.2%)*	*36.5%*
Maternal Age					
	<20	*11,225 (8.3%)*	*55.3%*	*9,083 (11.6%)*	*44.7%*
	20-24	*26,889 (19.9%)*	*57.7%*	*19,700 (25.1%)*	*42.3%*
	25-29	*40,927 (30.3%)*	*64.3%*	*22,758 (29.0%)*	*35.7%*
	30-34	*37,314 (27.6%)*	*67.3%*	*18,160 (23.1%)*	*32.7%*
	≥35	*18,784 (13.9%)*	*68.0%*	*8,821 (11.2%)*	*32.0%*
Feeding Method(At Birth)					
	Exclusive	*71,291 (52.8%)*	*65.9%*	*36,856 (46.9%)*	*34.1%*
	Partial	*38,205 (28.3%)*	*62.7%*	*22,686 (28.9%)*	*37.3%*
	Formula Fed	*23,556 (17.4%)*	*57.4%*	*17,470 (22.2%)*	*42.6%*
	Unknown	*2,087 (1.5%)*	*58.0%*	*1,510 (1.9%)*	*42.0%*
Multiple Birth					
	No	*133,308 (98.6%)*	*63.3%*	*77,403 (98.6%)*	*36.7%*
	Yes	*1,831 (1.4%)*	*62.1%*	*1,119 (1.4%)*	*37.9%*
Parity					
	0	*52,859 (39.1%)*	*65.4%*	*27,952 (35.6%)*	*34.6%*

	1	*44,364 (32.8%)*	*64.0%*	*24,929 (31.7%)*	*36.0%*
	2	*20,427 (15.1%)*	*60.6%*	*13,272 (16.9%)*	*39.4%*
	3	*8,554 (6.3%)*	*58.6%*	*6,043 (7.7%)*	*41.4%*
	≥4	*8,459 (6.3%)*	*58.4%*	*6,022 (7.7%)*	*41.6%*
	Unknown	*476 (0.4%)*	*61.0%*	*304 (0.4%)*	*39.0%*
Number of Children In Household					
	1	*52,886 (39.1%)*	*65.3%*	*28,053 (35.7%)*	*34.7%*
	2	*45,265 (33.5%)*	*63.4%*	*26,138 (33.3%)*	*36.6%*
	3	*20,719 (15.3%)*	*60.5%*	*13,515 (17.2%)*	*39.5%*
	≥4	*16,144 (11.9%)*	*60.1%*	*10,698 (13.6%)*	*39.9%*
	Unknown	*125 (0.1%)*	*51.4%*	*118 (0.2%)*	*48.6%*
Year Of Birth					
	1996-1999	*31,851 (23.6%)*	*62.6%*	*19,058 (24.3%)*	*37.4%*
	2000-2003	*30,153 (22.3%)*	*63.1%*	*17,663 (22.5%)*	*36.9%*
	2004-2007	*31,447 (23.3%)*	*64.2%*	*17,563 (22.4%)*	*35.8%*
	2008-2012	*41,688 (30.8%)*	*63.2%*	*24,238 (30.9%)*	*36.8%*
Season Of Birth					
	Winter	*31,540 (23.3%)*	*63.5%*	*18,155 (23.1%)*	*36.5%*
	Spring	*34,271 (25.4%)*	*62.9%*	*20,200 (25.7%)*	*37.1%*
	Summer	*35,333 (26.1%)*	*62.9%*	*20,867 (26.6%)*	*37.1%*
	Fall	*33,995 (25.2%)*	*63.8%*	*19,300 (24.6%)*	*36.2%*
Maternal Hospital Utilization					
	No	*70,952 (52.5%)*	*64.5%*	*39,071 (49.8%)*	*35.5%*
	Yes	*64,187 (47.5%)*	*61.9%*	*39,451 (50.2%)*	*38.1%*
Maternal Physician Utilization					
	<6	*43,345 (32.1%)*	*70.3%*	*18,323 (23.3%)*	*29.7%*
	6 to 12	*29,474 (21.8%)*	*63.9%*	*16,661 (21.2%)*	*36.1%*
	13 to 28	*34,252 (25.3%)*	*63.0%*	*20,107 (25.6%)*	*37.0%*
	≥29	*28,068 (20.8%)*	*54.5%*	*23,431 (29.8%)*	*45.5%*

* Proportion within the antenatal antibiotic exposed and non-exposed groups for each variable (Vertical).

$ Percentage across the antenatal antibiotic exposed and non-exposed groups for each variable (Horizontal)

3.2. Severe Allergy

Among the antenatal antibiotic exposed group, there were 1,479 children (IR 1.76 per 1000 person-years, 95% CI: 1.67-1.85) who had a positive outcome for severe allergy, while in the unexposed group it was 2,658 (IR 1.85 per 1000 person-years, 95% CI: 1.78-1.92). The incidence rate ratio of 0.95 (CI: 0.89-1.02) was not indicative of a significant difference between the incidence rate in the exposed and non-exposed group.

Severe allergy in children was significantly associated with maternal severe allergy (HR 2.82; 95% CI: 1.96-4.06), male gender (HR 1.46; 95% CI: 1.38-1.56), urban residence (HR 2.22; 95% CI: 2.07-2.38) and C-section delivery (HR 1.24; 95% CI: 1.15-1.34) as represented in Table 3. Maternal age greater than 24 years at parturition increased the risk of severe allergy in children, with the highest risk being maternal age >35 years (HR 2.24; 95% CI: 2.01-2.50). Children who were formula fed at birth remarkably had reduced odds (HR 0.63; 95% CI: 0.57-0.69) for the development of severe allergy in comparison to children who were exclusively breastfed. However, partially breastfed children had narrowly increased risk (HR 1.14; 95% CI: 1.07-1.22) for a positive outcome in the future. Gestational age and multiple births (HR 1.11; 95% CI: 0.86-1.42) were not found to increase the risk of severe allergy in the population. When the highest income quintile (Q5) was used as a reference, there was a steady decrease in the risk of severe allergy with each income quintile, with the lowest quintile (Q1) having the least risk (HR 0.50; 95% CI: 0.46-0.55). Risk of severe allergy in infants was found to decrease with increasing parity (HR 0.77; 95% CI: 0.74-0.79) and increasing number of children in the household (HR 0.74 95% CI: 0.71-0.76). There was an evident seasonal influence on the allergic outcome as children born in the fall and winter had higher allergic risk than those born in spring and summer. Post-natal

antibiotic administration in infants was associated with an increased risk for severe allergy, when the exposure was within one year from birth (HR: 1.50; 95%CI: 1.41-1.60).

Maternal Antibiotic Use:

Development of severe allergy during childhood was not associated with maternal antibiotic exposure during pregnancy period (HR 0.96; 95%CI: 0.90-1.02). However, after adjusting for covariates and post-natal antibiotic use (Table 5; Figure 1), the exposure was found to have a weak association with the outcome (aHR1.08; 95% CI: 1.01-1.15). The trimester-specific analysis for the temporal relation of antibiotic exposure with outcome found no association before and after adjustment for covariates. Macrolides, lincosamides, and streptogramins use during the antenatal period were associated (aHR 1.20; 95%CI: 1.07-1.35) with severe childhood allergy after sub-group analysis based on antibiotic type. None of the other classes of antibiotics were found to be associated with the disease outcome during childhood in the population. Although we did not see a strong dose-response relation for increasing severe childhood allergy with each added course of maternal antibiotic use during pregnancy, those exposed to 3 or more courses did have a significant association (aHR 1.17 95%CI:1.03-1.34). Post-pregnancy maternal antibiotic use had a similar connection as antenatal antibiotic use after adjusting for covariates and post-natal infant antibiotics (aHR 1.08 95%CI: 1.01-1.15). Remarkably, our study detected a relationship between pre-pregnancy antibiotic consumption in mothers and severe allergy outcome in the offspring after adjusting for covariates and post-natal infant antibiotic use (aHR 1.12 95%CI: 1.05-1.19). Severe allergy outcome in the child was not significantly associated with increasing maternal healthcare utilization (HR: 0.68; 95% CI:0.64-0.73) or physician visits.

Table 3: Severe Allergy Outcome by Covariates

	The child has severe allergy	
	No (N=209,524)	**Yes (N=4,137)**
Mother has Condition		
No	208,952 (99.7%)	4,108 (99.3%)
Yes	572 (0.3%)	29 (0.7%)
Gender		
Male	106,935 (51.0%)	2,502 (60.5%)
Female	102,589 (49.0%)	1,635 (39.5%)
Residence Locality (Urban or Rural)		
Urban	112,618 (53.7%)	2,986 (72.2%)
Rural	96,405 (46.0%)	1,144 (27.7%)
Unknown	501 (0.2%)	7 (0.2%)
Income Quintile		
Q1 (lowest)	55,223 (26.4%)	796 (19.2%)
Q2	43,397 (20.7%)	817 (19.7%)
Q3	39,066 (18.6%)	731 (17.7%)
Q4	38,187 (18.2%)	830 (20.1%)
Q5 (highest)	33,150 (15.8%)	956 (23.1%)
Unknown	501 (0.2%)	7 (0.2%)
Gestation Period (Weeks)		
<35	4,926 (2.4%)	90 (2.2%)
35 to <37	9,022 (4.3%)	170 (4.1%)
37 to <39	42,936 (20.5%)	863 (20.9%)
39+	152,640 (72.9%)	3,014 (72.9%)
Birth Weight (Grams)		
<3000	36,103 (17.2%)	730 (17.6%)
3000 to <3500	69,173 (33.0%)	1,346 (32.5%)
3500 to <4500	97,241 (46.4%)	1,908 (46.1%)
4500+	6,420 (3.1%)	< 152 (< 3.7%)
Unknown	587 (0.3%)	< 6 (< 0.1%)
Delivery Method		
Caesarean Section	40,315 (19.2%)	933 (22.6%)
Vaginal Delivery	169,209 (80.8%)	3,204 (77.4%)
Maternal Age		
<20	20,101 (9.6%)	207 (5.0%)
20-24	45,998 (22.0%)	591 (14.3%)
25-29	62,471 (29.8%)	1,214 (29.3%)
30-34	54,115 (25.8%)	1,359 (32.8%)
35+	26,839 (12.8%)	766 (18.5%)
Feeding Method (at birth)		
Fully Breastfed	105,930 (50.6%)	2,217 (53.6%)
Partially Breastfed	59,542 (28.4%)	1,349 (32.6%)
Formula Fed	40,502 (19.3%)	524 (12.7%)

Unknown	3,550 (1.7%)	47 (1.1%)
Multiple Birth		
No	206,637 (98.6%)	4,074 (98.5%)
Yes	2,887 (1.4%)	63 (1.5%)
Parity		
0	78,837 (37.6%)	1,974 (47.7%)
1	67,883 (32.4%)	1,410 (34.1%)
2	33,175 (15.8%)	524 (12.7%)
3	14,460 (6.9%)	137 (3.3%)
4+	14,393 (6.9%)	< 89 (< 2.2%)
Unknown	776 (0.4%)	< 6 (< 0.1%)
Number of Children in Household		
1	78,929 (37.7%)	2,010 (48.6%)
2	69,977 (33.4%)	1,426 (34.5%)
3	33,720 (16.1%)	514 (12.4%)
4+	26,656 (12.7%)	< 187 (< 4.5%)
Unknown	242 (0.1%)	< 6 (< 0.1%)
Maternal Pre-pregnancy Exposure		
No	135,853 (64.8%)	2,642 (63.9%)
Yes	73,671 (35.2%)	1,495 (36.1%)
Maternal Post-pregnancy Exposure		
No	135,227 (64.5%)	2,673 (64.6%)
Yes	74,297 (35.5%)	1,464 (35.4%)
Antenatal Exposure		
No	132,481 (63.2%)	2,658 (64.2%)
Yes	77,043 (36.8%)	1,479 (35.8%)
Total Number of Courses		
None	132,481 (63.2%)	2,658 (64.2%)
1	46,369 (22.1%)	917 (22.2%)
2	17,633 (8.4%)	321 (7.8%)
3+	13,041 (6.2%)	241 (5.8%)
Beta-lactam antibacterials, penicillins		
Unexposed	157,891 (75.4%)	3,172 (76.7%)
Exposed	51,633 (24.6%)	965 (23.3%)
Other beta-lactam antibacterials		
Unexposed	196,719 (93.9%)	3,944 (95.3%)
Exposed	12,805 (6.1%)	193 (4.7%)
Macrolides, lincosamides and streptogramins		
Unexposed	194,734 (92.9%)	3,807 (92.0%)
Exposed	14,790 (7.1%)	330 (8.0%)
Tetracyclines, Aminoglycosides, Quinolones, and other antibacterials		
Unexposed	193,759 (92.5%)	3,828 (92.5%)
Exposed	15,765 (7.5%)	309 (7.5%)
Sulphonamides and Trimethoprim		
Unexposed	204,001 (97.4%)	4,045 (97.8%)
Exposed	5,523 (2.6%)	92 (2.2%)

Trimester 1		
Unexposed	175,623 (83.8%)	3,476 (84.0%)
Exposed	33,901 (16.2%)	661 (16.0%)
Trimester 2		
Unexposed	170,902 (81.6%)	3,419 (82.6%)
Exposed	38,622 (18.4%)	718 (17.4%)
Trimester 3		
Unexposed	178,757 (85.3%)	3,574 (86.4%)
Exposed	30,767 (14.7%)	563 (13.6%)
0-3 Months		
Unexposed	199,397 (95.2%)	3,906 (94.4%)
Exposed	10,127 (4.8%)	231 (5.6%)
3-6 Months		
Unexposed	178,885 (85.4%)	3,400 (82.2%)
Exposed	30,639 (14.6%)	737 (17.8%)
6-12 Months		
Unexposed	126,316 (60.3%)	2,358 (57.0%)
Exposed	83,208 (39.7%)	1,779 (43.0%)
Birth to 1 Year		
Unexposed	113,679 (54.3%)	2,057 (49.7%)
Exposed	95,845 (45.7%)	2,080 (50.3%)
1+ Years		
Unexposed	33,895 (16.2%)	1,316 (31.8%)
Exposed	175,629 (83.8%)	2,821 (68.2%)
Year of Birth		
1996-1999	49,644 (23.7%)	1,265 (30.6%)
2000-2003	46,555 (22.2%)	1,261 (30.5%)
2004-2007	47,838 (22.8%)	1,172 (28.3%)
2008-2012	65,487 (31.3%)	439 (10.6%)
Month of Birth		
Winter	48,560 (23.2%)	1,135 (27.4%)
Spring	53,563 (25.6%)	908 (21.9%)
Summer	55,222 (26.4%)	978 (23.6%)
Fall	52,179 (24.9%)	1,116 (27.0%)
Age at Diagnosis		
<5	*N/A*	2,937 (71.0%)
5 to <10	*N/A*	884 (21.4%)
10 to <15	*N/A*	299 (7.2%)
15+	*N/A*	17 (0.4%)
Child's Hospital Utilization		
No	168,046 (80.2%)	1,018 (24.6%)
Yes	41,478 (19.8%)	3,119 (75.4%)
Child's Physician Utilization		
<6	56,934 (27.2%)	92 (2.2%)
6 to 10	53,077 (25.3%)	350 (8.5%)
11 to 18	54,297 (25.9%)	1,115 (27.0%)

19+	45,216 (21.6%)	2,580 (62.4%)
Mother's Hospital Utilization		
No	107,466 (51.3%)	2,557 (61.8%)
Yes	102,058 (48.7%)	1,580 (38.2%)
Mother's Physician Utilization		
<6	60,457 (28.9%)	1,211 (29.3%)
6 to 12	45,103 (21.5%)	1,032 (24.9%)
13 to 28	53,321 (25.4%)	1,038 (25.1%)
29+	50,643 (24.2%)	856 (20.7%)

Table 4. Probable confounding influences in maternal antibiotic exposure and severe childhood allergy

Characteristic		**Antenatal antibiotic**		**Severe childhood allergy**	
	N	**N**	**%**	**N**	**HR (95%CI)**
All dyads	213,661	78,522	36.8%	4,137	n/a
Antenatal antibiotic use					
Yes	78,522	NA	NA	1,479	0.96 (0.90,1.02)
No	135,139	NA	NA	2,658	1.00 (ref)
Infant antibiotic use before 12 months					
Yes	97,925	42,903	43.8%	2,080	1.50 (1.41,1.60)
No	115,736	35,619	30.8%		1.00 (ref)
Infant Sex					
Male	109,437	40,267	36.8%	2,502	1.46 (1.38,1.56)
Female	104,224	38,255	36.7%	1,635	1.00 (ref)
Residence Location					
Urban	115,604	40,246	34.8%	2,986	2.22 (2.07,2.38)
Rural	97,549	38,056	39.0%	1,144	1.00 (ref)
Gestation Period (Weeks)					
<35	5,016	1,962	39.1%	90	0.93 (0.75,1.14)
35 to <37	9,192	3,594	39.1%	170	0.97 (0.83,1.13)
37 to <39	43,799	16,429	37.5%	863	1.03 (0.96,1.12)
39+	155,654	56,537	36.3%	3,014	1.00 (ref)
Income Quintile					
Quintile 1 (low)	56,019	23,426	41.8%	796	0.50 (0.46,0.55)
Quintile 2	44,214	16,637	37.6%	817	0.65 (0.60,0.72)
Quintile 3	39,797	14,499	36.4%	731	0.65 (0.59,0.72)
Quintile 4	39,017	12,962	33.2%	830	0.76 (0.69,0.83)
Quintile 5 (high)	34,106	10,778	31.6%	956	1.00 (ref)
Multiple Birth					
Yes	2,950	1,119	37.9%	63	1.12 (0.88,1.44)
No	210,711	77,403	36.7%	4074	1.00 (ref)

Maternal Age					
<20	20,308	9,083	44.7%	207	0.80 (0.68,0.93)
20-24	46,589	19,700	42.3%	591	1.00 (ref)
25-29	63,685	22,758	35.7%	1,214	1.52 (1.38,1.67)
30-34	55,474	17,160	30.9%	1,359	1.96 (1.78,2.16)
35+	27,605	8,821	32.0%	766	2.24 (2.01,2.50)
Delivery Method					
Caesarean Section	41,248	15,557	37.7%	933	1.24 (1.15,1.34)
Vaginal	172,413	62,965	36.5%	3,204	1.00 (ref)
Birth Weight (grams)					
<3000	36,833	13,640	37.0%	730	0.87 (0.73,1.04)
3000 to <3500	70,519	25,353	36.0%	1,346	0.84 (0.71,0.99)
3500 to <4500	99,149	36,626	36.9%	1,908	0.84 (0.71,0.99)
4500+	6,571	2,613	39.8%	152	1.00 (ref)
Maternal Physician Utilization					
<6	61,668	18,323	29.7%	1,211	1.00 (ref)
6 to 12	46,135	16,661	36.1%	1,032	1.15 (1.06,1.25)
13 to 28	54,359	20,107	37.0%	1,038	1.01 (0.93,1.09)
≥29	51,499	23,431	45.5%	856	0.92 (0.82,1.00)
Maternal Hospital Utilization					
Yes	103,638	39,451	38.1%	1,580	0.68 (0.64,0.73)
No	110,023	39,071	35.5%	2,557	1.00 (ref)

HR, hazard ratio; CI, confidence interval.
[$] Significantly increased risk at P-value <0.05

Table 5. Unadjusted and adjusted estimates of the association between maternal and infant antibiotic use and severe allergy in the child.

Antibiotic exposure	**Unadjusted HR**	**Adjusted for *a priori* Covariates***	**Adjusted for *a priori* Covariates* + Both Maternal and Infant Antibiotics**
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Any antenatal antibiotic exposure			
No	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	0.96 (0.90-1.02)	1.01 (0.95-1.08)	1.08 (1.01-1.15)
Number of antenatal antibiotic courses			
0 (Nil)	1.00 (ref)	1.00 (ref)	1.00 (ref)
1	0.99 (0.91-1.06)	1.01 (0.94-1.09)	1.07 (0.99-1.15)
2	0.91 (0.81-1.02)	0.98 (0.87-1.10)	1.05 (0.94-1.18)
≥3	0.92 (0.80-1.04)	1.06 (0.93-1.21)	1.17 (1.03-1.34)
Type of antibiotic in the antenatal period			
Beta-lactams, penicillins	0.93 (0.86-1.00)	0.98 (0.91-1.05)	1.05 (0.98-1.13)
Beta-lactams, others	0.80 (0.69-0.93)	0.93 (0.80-1.08)	0.93 (0.80-1.07)

Macrolides, streptogramins, lincosamides	1.16 (1.03-1.30)[$]	1.16 (1.03-1.30)[$]	1.20 (1.07-1.35)
Tetracyclines, aminoglycosides, quinolones	1.05 (0.94-1.18)	1.04 (0.92-1.17)	1.05 (0.93-1.18)
Sulphonamides and trimethoprim	0.82 (0.66-1.01)	0.86 (0.70-1.06)	0.90 (0.73-1.11)
Timing of maternal antibiotic use			
9 months before conception	1.04 (0.98-1.11)	1.04 (0.97 - 1.11)	1.12 (1.05 - 1.19)
Trimester 1	1.01 (0.93-1.10)	1.02 (0.93-1.11)	1.06 (0.97-1.15)
Trimester 2	0.94 (0.87-1.02)	0.98 (0.90-1.06)	1.03 (0.95-1.12)
Trimester 3	0.92 (0.84-1.00)	1.02 (0.93-1.12)	1.06 (0.97-1.16)
9 months post-partum	0.99 (0.93 - 1.06)	1.01 (0.95-1.08)	1.08 (1.01 - 1.15)

HR, hazard ratio; CI, confidence interval. *Covariates: maternal chronic disease, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

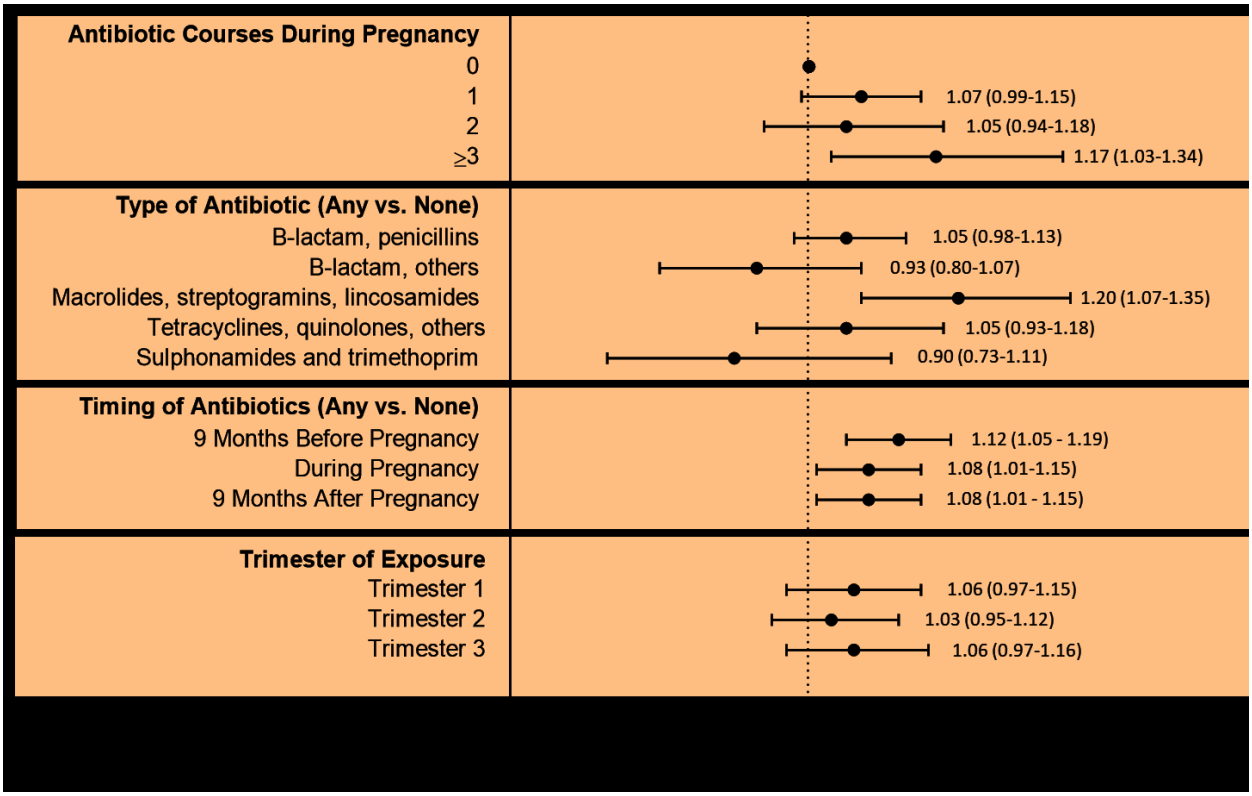


Figure 1: Associations between maternal antibiotics during pregnancy and severe allergy in their offspring by number of antibiotic courses, type of antibiotic, timing of exposure and trimester of exposure.

Adjust for confounders: maternal severe allergy, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

3.3. Celiac Disease

2,540 children had a positive outcome for Celiac disease in Manitoba from 1996-2012, which accounted for 1.4% of the study population. Maternal antibiotic exposure during pregnancy was positive for 1,059 children (IR 1.26 per 1,000 person-years; CI: 1.18-1.34) and negative for 1,481 children (IR 1.02 per 1,000 person-years; CI: 0.97-1.08). The incidence rate ratio of 1.23 (CI: 1.14-1.33) was indicative of a significant difference between the incidence rate in the exposed and non-exposed group. A child whose mother had celiac disease had a significantly higher risk (HR 6.84 CI: 4.45-10.51) of developing celiac disease than the child of a disease-naïve mother (Table 6). Male gender was not associated with a significantly elevated risk of celiac diseases. Although the residence in an urban locality was associated with an increased risk (HR 1.32 CI: 1.21-1.42) of childhood celiac disease development, the income quintile of the family did not have any influence on the outcome.

In comparison to term pregnancy, especially beyond 39 weeks, preterm pregnancy <35 weeks was associated with the highest risk (HR 1.43 CI: 1.15-1.79) of celiac disease development. While the risk of celiac disease was higher (HR 1.25 CI: 1.14-1.38) for children born through cesarean section, neither the maternal age at parturition nor the birth weight of child has any obvious influence on the outcome.

Similarly, multiple births (HR: 0.75 CI: 0.51-1.10) and feeding method (in hospital) was not found to influence the development of childhood celiac disease. Parity>0 and number of children in the household >1 in-house were found to have a protective influence on the disease outcome with HR of 0.91 (0.89-0.94) and 0.85 (0.82-0.88) respectively.

Children who had direct antibiotic exposure in the first 3 post-natal months had a higher risk of disease development (HR: 1.61 CI: 1.36-1.92) than unexposed children. The association became weaker to absent for exposures between 3-6 months and 6-12 months respectively. When the risk for the first post-natal year was combined, there was only a marginal elevation in the risk for disease development (HR: 1.23 CI: 1.13-1.34). While maternal hospital utilization did not influence the outcome, maternal physician consultation >6 marginally increased the risk of disease development in offspring.

Maternal antibiotics were associated with an increased risk of gluten allergy/celiac disease in offspring irrespective of the timing of exposure concerning pregnancy (Table 7; Figure 2). After adjusting for covariates, postpartum maternal antibiotic posed the highest risk (aHR: 1.77 CI: 1.63-1.92), followed by pre-pregnancy exposure (aHR: 1.68 CI: 1.55-1.82) and antenatal exposure (aHR: 1.59 CI: 1.46-1.71). Number of antibiotic courses ≥3 during pregnancy was associated with a relatively higher risk (HR: 1.54 CI: 1.34-1.77) of celiac disease in offspring, and this dose-response relationship became stronger after adjusting for covariates. There was no trimester-specific temporal relation between antibiotic exposure and outcome as evident from the lack of any trimester specific increase in risk. Maternal use of beta-lactam penicillins during pregnancy significantly increased the risk (HR: 1.27 CI: 1.17-1.39) of celiac disease in offspring (the significance associated with tetracycline, quinolones & others was negligible). However, after adjusting for covariates, all antibiotic groups except “other beta-lactams” displayed at least a borderline significance. Beta-lactam penicillin use was associated with the highest risk among all antibiotics with an aHR of 1.65 (1.51-1.80).

Table 6: Probable confounding influences in maternal antibiotic exposure and childhood celiac disease

		Childhood Celiac Disease		
		No	***Yes***	***Crude Hazards Ratio (95% CI)***
		(N=211,121)	***(N=2,540)***	
Mother has Condition				
	No	*210,852 (99.9%)*	*2,519 (99.2%)*	*1 (ref)*
	Yes	*269 (0.1%)*	*21 (0.8%)*	*6.84 (4.45-10.51)*
Gender				
	Male	*108,088 (51.2%)*	*1,349 (53.1%)*	*1.08 (1.00-1.17)*
	Female	*103,033 (48.8%)*	*1,191 (46.9%)*	*1 (ref)*
Residence Locality				
	Urban	*114,059 (54.0%)*	*1,545 (60.8%)*	*1.32 (1.21-1.42)*
	Rural	*96,559 (45.7%)*	*991 (< 39.0%)*	*1 (ref)*
	Unknown	*503 (0.2%)*	*< 6 (< 0.2%)*	*1.01 (0.42-2.43)*
Income Quintile				
	Q1 (lowest)	*55,411 (26.2%)*	*608 (23.9%)*	*0.92 (0.81-1.04)*
	Q2	*43,640 (20.7%)*	*574 (22.6%)*	*1.09 (0.96-1.24)*
	Q3	*39,321 (18.6%)*	*476 (18.7%)*	*1.01 (0.88-1.15)*
	Q4	*38,546 (18.3%)*	*471 (18.5%)*	*1.02 (0.89-1.16)*
	Q5 (highest)	*33,700 (16.0%)*	*< 407 (< 16.0%)*	*1 (ref)*
	Unknown	*503 (0.2%)*	*< 6 (< 0.2%)*	*0.86 (0.36-2.09)*
Gestation Period (Weeks)				
	<35	*4,935 (2.3%)*	*81 (3.2%)*	*1.43 (1.15-1.79)*
	35 to <37	*9,073 (4.3%)*	*119 (4.7%)*	*1.16 (0.96-1.39)*
	37 to <39	*43,228 (20.5%)*	*571 (22.5%)*	*1.17 (1.06-1.28)*
	39+	*153,885 (72.9%)*	*1,769 (69.6%)*	*1 (ref)*
Birth Weight (Grams)				
	<3000	*36,317 (17.2%)*	*516 (20.3%)*	*1.29 (1.01-1.65)*
	3000 to <3500	*69,698 (33.0%)*	*821 (32.3%)*	*1.07 (0.84-1.36)*
	3500 to <4500	*98,023 (46.4%)*	*1,126 (44.3%)*	*1.04 (0.82-1.32)*

	4500+	*6,499 (3.1%)*	*< 73 (< 2.9%)*	*1 (ref)*
	Unknown	*584 (0.3%)*	*< 6 (< 0.2%)*	*0.92 (0.37-2.27)*
Delivery Method				
	C- Section	*40,670 (19.3%)*	*578 (22.8%)*	*1.25 (1.14-1.38)*
	Vaginal	*170,451 (80.7%)*	*1,962 (77.2%)*	*1 (ref)*
Maternal Age				
	<20	*20,074 (9.5%)*	*234 (9.2%)*	*1.04 (0.89-1.21)*
	20-24	*46,075 (21.8%)*	*514 (20.2%)*	*1 (ref)*
	25-29	*62,957 (29.8%)*	*728 (28.7%)*	*1.04 (0.93-1.16)*
	30-34	*54,755 (25.9%)*	*719 (28.3%)*	*1.18 (1.05-1.32)*
	35+	*27,260 (12.9%)*	*345 (13.6%)*	*1.15 (1.00-1.31)*
Feeding Method (at birth)				
	Exclusive	*106,821 (50.6%)*	*1,326 (52.2%)*	*1 (ref)*
	Partially	*60,221 (28.5%)*	*670 (26.4%)*	*0.95 (0.87-1.05)*
	Formula Fed	*40,535 (19.2%)*	*491 (19.3%)*	*0.99 (0.89-1.10)*
	Unknown	*3,544 (1.7%)*	*53 (2.1%)*	*1.22 (0.93-1.60)*
Multiple Birth				
	No	*208,197 (98.6%)*	*2,514 (99.0%)*	*1 (ref)*
	Yes	*2,924 (1.4%)*	*26 (1.0%)*	*0.75 (0.51-1.10)*
Parity				
	0	*79,684 (37.7%)*	*1,127 (44.4%)*	*0.91 (0.89-0.94)*
	1	*68,478 (32.4%)*	*815 (32.1%)*	
	2	*33,344 (15.8%)*	*355 (14.0%)*	
	3	*14,480 (6.9%)*	*< 118 (< 4.6%)*	
	≥4	*14,358 (6.8%)*	*123 (4.8%)*	
	Unknown	*777 (0.4%)*	*< 6 (< 0.2%)*	
Number of Children in Household				
	1	*79,794 (37.8%)*	*1,145 (45.1%)*	*0.85 (0.82-0.88)*
	2	*70,558 (33.4%)*	*845 (33.3%)*	
	3	*33,879 (16.0%)*	*355 (14.0%)*	
	≥4	*26,650 (12.6%)*	*< 193 (< 7.6%)*	

	Unknown	*240 (0.1%)*	*< 6 (< 0.2%)*	
Maternal Pre-pregnancy Exposure				
	No	*137,006 (64.9%)*	*1,489 (58.6%)*	*1 (ref)*
	Yes	*74,115 (35.1%)*	*1,051 (41.4%)*	*1.30 (1.20-1.41)*
Maternal Post-pregnancy Exposure				
	No	*136,444 (64.6%)*	*1,456 (57.3%)*	*1 (ref)*
	Yes	*74,677 (35.4%)*	*1,084 (42.7%)*	*1.35 (1.25-1.46)*
Antenatal Exposure				
	No	*133,658 (63.3%)*	*1,481 (58.3%)*	*1 (ref)*
	Yes	*77,463 (36.7%)*	*1,059 (41.7%)*	*1.23 (1.14-1.33)*
Total Number of Courses				
	None	*133,658 (63.3%)*	*1,481 (58.3%)*	*1 (ref)*
	1	*46,666 (22.1%)*	*620 (24.4%)*	*1.20 (1.09-1.32)*
	2	*17,739 (8.4%)*	*215 (8.5%)*	*1.09 (0.95-1.26)*
	≥3	*13,058 (6.2%)*	*224 (8.8%)*	*1.54 (1.34-1.77)*
J01C				
	Unexposed	*159,274 (75.4%)*	*1,789 (70.4%)*	*1 (ref)*
	Exposed	*51,847 (24.6%)*	*751 (29.6%)*	*1.27 (1.17-1.39)*
J01D				
	Unexposed	*198,262 (93.9%)*	*2,401 (94.5%)*	*1 (ref)*
	Exposed	*12,859 (6.1%)*	*139 (5.5%)*	*0.94 (0.79-1.11)*
J01F				
	Unexposed	*196,207 (92.9%)*	*2,334 (91.9%)*	*1 (ref)*
	Exposed	*14,914 (7.1%)*	*206 (8.1%)*	*1.16 (1.00-1.33)*
J01A, J01M, J01X				
	Unexposed	*195,266 (92.5%)*	*2,321 (91.4%)*	*1 (ref)*
	Exposed	*15,855 (7.5%)*	*219 (8.6%)*	*1.18 (1.03-1.36)*
J01E				
	Unexposed	*205,585 (97.4%)*	*2,461 (96.9%)*	*1 (ref)*
	Exposed	*5,536 (2.6%)*	*79 (3.1%)*	*1.15 (0.92-1.44)*
Trimester 1				

Trimester 2	*Unexposed*	*177,057 (83.9%)*	*2,042 (80.4%)*	*1 (ref)*
	Exposed	*34,064 (16.1%)*	*498 (19.6%)*	*1.23 (1.11-1.35)*
Trimester 3	*Unexposed*	*172,321 (81.6%)*	*2,000 (78.7%)*	*1 (ref)*
	Exposed	*38,800 (18.4%)*	*540 (21.3%)*	*1.14 (1.04-1.26)*
0-3 Months	*Unexposed*	*180,213 (85.4%)*	*2,118 (83.4%)*	*1 (ref)*
	Exposed	*30,908 (14.6%)*	*422 (16.6%)*	*1.09 (0.98-1.21)*
3-6 Months	*Unexposed*	*200,959 (95.2%)*	*2,396 (94.3%)*	*1 (ref)*
	Exposed	*10,162 (4.8%)*	*144 (5.7%)*	*1.61 (1.36-1.92)*
6-12 Months	*Unexposed*	*180,239 (85.4%)*	*2,229 (87.8%)*	*1 (ref)*
	Exposed	*30,882 (14.6%)*	*311 (12.2%)*	*1.15 (1.01-1.30)*
Birth to 1 Year	*Unexposed*	*127,360 (60.3%)*	*1,776 (69.9%)*	*1 (ref)*
	Exposed	*83,761 (39.7%)*	*764 (30.1%)*	*1.07 (0.98-1.18)*
>1 Years	*Unexposed*	*114,609 (54.3%)*	*1,589 (62.6%)*	*1 (ref)*
	Exposed	*96,512 (45.7%)*	*951 (37.4%)*	*1.23 (1.13-1.34)*
Year of Birth	*Unexposed*	*33,914 (16.1%)*	*1,445 (56.9%)*	*1 (ref)*
	Exposed	*177,207 (83.9%)*	*1,095 (43.1%)*	*0.11 (0.1-0.12)*
	1996-1999	*50,005 (23.7%)*	*904 (35.6%)*	*0.89 (0.80-0.98)*
	2000-2003	*47,114 (22.3%)*	*702 (27.6%)*	*0.71 (0.64-0.80)*
	2004-2007	*48,491 (23.0%)*	*519 (20.4%)*	*0.52 (0.46-0.58)*
	2008-2012	*65,511 (31.0%)*	*415 (16.3%)*	*1 (ref)*
Season of Birth	*Winter*	*49,062 (23.2%)*	*633 (24.9%)*	*1.05 (0.95-1.17)*
	Spring	*53,857 (25.5%)*	*614 (24.2%)*	*0.93 (0.84-1.04)*

	Summer	*55,529 (26.3%)*	*671 (26.4%)*	*1 (ref)*
	Fall	*52,673 (24.9%)*	*622 (24.5%)*	*0.99 (0.89-1.10)*
Child's Hospital Utilization				
	No	*169,650 (80.4%)*	*545 (21.5%)*	
	Yes	*41,471 (19.6%)*	*1,995 (78.5%)*	*23.54 (21.34-25.98)*
Child's Physician Utilization				
	<6	*56,917 (27.0%)*	*552 (21.7%)*	*1 (ref)*
	6 to 10	*53,404 (25.3%)*	*430 (16.9%)*	*0.86 (0.76-0.98)*
	11 to 18	*54,963 (26.0%)*	*557 (21.9%)*	*1.14 (1.01-1.28)*
	19+	*45,837 (21.7%)*	*1,001 (39.4%)*	*2.44 (2.20-2.71)*
Mother's Hospital Utilization				
	No	*108,534 (51.4%)*	*1,489 (58.6%)*	*1 (ref)*
	Yes	*102,587 (48.6%)*	*1,051 (41.4%)*	*0.79 (0.73-0.85)*
Mother's Physician Utilization				
	<6	*61,046 (28.9%)*	*622 (24.5%)*	*1 (ref)*
	6 to 12	*45,499 (21.6%)*	*636 (25.0%)*	*1.39 (1.24-1.55)*
	13 to 28	*53,700 (25.4%)*	*659 (25.9%)*	*1.26 (1.13-1.40)*
	29+	*50,876 (24.1%)*	*623 (24.5%)*	*1.33 (1.19-1.48)*

Table 7: Sensitivity analysis (Outcome: Celiac Disease)

	Crude HR HR (95% CI)	*Adjusted for Covariates* HR (95% CI)*	*Adjusted for Covariates* + Infant Antibiotics HR (95% CI)*
Timing of antibiotic use			
Pre-Pregnancy	*1.30 (1.20-1.41)*	*1.30 (1.20-1.41)*	*1.68 (1.55-1.82)*
Antenatal	*1.23 (1.14-1.33)*	*1.26 (1.16-1.36)*	*1.59 (1.46-1.71)*
Postnatal	*1.35 (1.25-1.46)*	*1.36 (1.26-1.47)*	*1.77 (1.63-1.92)*
Number of antibiotic courses during pregnancy			
1	*1.20 (1.09-1.32)*	*1.21 (1.10-1.33)*	*1.45 (1.32-1.59)*
2	*1.09 (0.95-1.26)*	*1.13 (0.98-1.30)*	*1.49 (1.29-1.72)*
≥3	*1.54 (1.34-1.77)*	*1.62 (1.41-1.87)*	*2.40 (2.08-2.77)*
Timing of exposure in pregnancy			
Trimester 1	*1.23 (1.11-1.35)*	*1.23 (1.11-1.36)*	*1.44 (1.30-1.60)*
Trimester 2	*1.14 (1.04-1.26)*	*1.16 (1.05-1.28)*	*1.38 (1.24-1.52)*
Trimester 3	*1.09 (0.98-1.21)*	*1.14 (1.03-1.27)*	*1.33 (1.19-1.48)*
Type of antibiotics exposed			
J01C	*1.27 (1.17-1.39)*	*1.30 (1.19-1.42)*	*1.65 (1.51-1.80)*
J01D	*0.94 (0.79-1.11)*	*0.92 (0.77-1.09)*	*0.94 (0.78-1.12)*
J01F	*1.16 (1.00-1.33)*	*1.12 (0.97-1.29)*	*1.30 (1.12-1.50)*
J01(A, M, X)	*1.18 (1.03-1.36)*	*1.12 (0.98-1.29)*	*1.19 (1.03-1.37)*
J01E	*1.15 (0.92-1.44)*	*1.08 (0.86-1.36)*	*1.32 (1.05-1.66)*

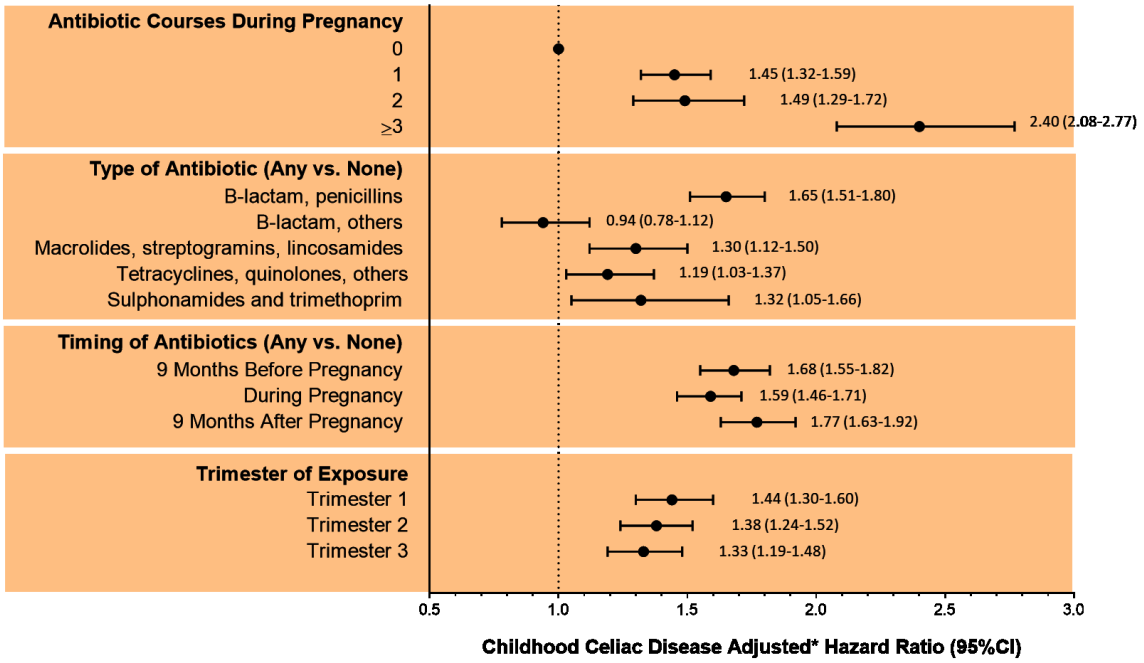


Figure 2: Associations between maternal antibiotics during pregnancy and celiac disease in their offspring by number of antibiotic courses, type of antibiotic, timing of exposure and trimester of exposure.

Adjust for confounders: maternal celiac disease, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

3.4. Type I Diabetes Mellitus

Between 1996 and 2012, there were 377 children who were diagnosed as having T1DM. Antenatal maternal antibiotic exposure was positive among 155 T1DM children (IR: 0.18 per 1,000 person-years; CI: 0.16-0.21) and negative for 222 children (IR: 0.15 per 1,000 person-years; CI: 0.13-0.17). The risk of T1DM development among maternal antibiotic exposed children was not significant with an IRR of 1.20 (CI: 0.97-1.47; Table 8). The even-rate of T1DM among children born to mothers with any form of diabetes was not found to be significantly different from children born to non-diabetic mothers (HR: 1.58 CI: 0.97-2.58). In comparison to female children who were used as the reference and assigned an HR of 1, male children were found to be at no higher risk (HR: 1.11 CI: 0.90-1.36). However, concerning rural residence, children who were born to families living in urban locality had a slightly higher risk for T1DM development (HR: 1.25 CI: 1.02-1.53). Concerning the highest income quintile, children born to families with lower incomes were not having a significantly higher risk of disease development. However, birth in families with the lowest income quintile had around 50% risk reduction (HR: 0.54 CI: 0.38-0.76) compared to birth in higher income families. Children who were born before 35 weeks of gestational age did not have a significantly higher event rate in comparison to the children who were born full-term.

Similarly, birth weight was also not found to influence the child's risk of T1DM development. The risk associated with C-section delivery was not found to be significantly different (HR: 1.04 CI: 0.80-1.34) from children born vaginally. Compared to children born to mothers aged between 20-24 years, which was used as a reference and assigned hazards ratio of 1, children born to mothers aged 30-34 years had a significantly higher event rate

(HR: 1.77 CI: 1.32-2.39). No difference in risk was evident among the children concerning the feeding method initiated at the hospital. There were only 6 children who were born as one of a multiple birth and developed T1DM in the population, and they had no greater risk (HR 0.80 CI: 0.30-2.15) than a singleton. A similar risk reduction of around 20% was observed among children with siblings (HR: 0.80 CI: 0.75-0.90) or born in households having children (HR: 0.84 CI: 0.77-0.92). Children who had postnatal antibiotic exposure between birth and 1 year age (HR 1.28 CI: 1.03-1.58), as well as exposure at age greater than 1 year of age (HR: 1.26 CI: 1.02-1.56), were associated with a higher even-rate in comparison to unexposed children. Neither the year of birth or the season of birth was found to be associated with an increased event rate for TIDM among children. Surprisingly, in comparison to children whose mothers did not utilize hospital services, there was a 26% risk reduction of T1DM among children whose mothers utilized hospital services. In comparison to children born to mothers who had physician consultation <6, the even-rate was not found to increase with increased physician utilization.

Children born to mothers who were exposed to antibiotics during pregnancy period did not have a significantly higher risk (HR: 1.20 CI: 0.98-1.47) of developing T1DM than children of non-exposed mothers and the risk remained similar after adjusting for *a priori* covariates and post-natal antibiotic use in children (HR: 1.22 CI: 0.99-1.51). Maternal antibiotic exposure before pregnancy and after pregnancy was not associated with an increased event rate in children before and after adjustment of covariates and post-natal antibiotic use in children (Table 9). A single course of antibiotic during pregnancy was associated with a higher risk of T1DM in childen, but the lower confidence interval was only marginally greater than 1. The marginal association remained unchanged after

adjusting for the covariates (HR: 1.36 CI: 1.08-1.72). There was no higher risk associated with more than one course of antibiotic indicating the absence of a dose-response relationship. Similarly, there was lack of a perceivable temporal gradience as the risk of T1DM was not higher for any of the trimesters before and after adjusting for *a priori* covariates. Though the penicillin and macrolide, streptogramins & lincosamide group had a greater hazards ratio than the other antibiotic groups (both before and after adjustment), the risk was not significant in both groups.

Table 8: Probable confounding influences in maternal antibiotic exposure and childhood type 1 diabetes mellitus

		Childhood Type 1 Diabetes Mellitus		
		No	***Yes***	***Crude Hazards Ratio (95% CI)***
		(N=213,284)	***(N=377)***	
Maternal Diabetes (any form)				
	No	*204,734 (96.0%)*	*360 (95.5%)*	*1 (ref)*
	Yes	*8,550 (4.0%)*	*17 (4.5%)*	*1.58 (0.97-2.58)*
Gender				
	Male	*109,234 (51.2%)*	*203 (53.8%)*	*1.11 (0.90-1.36)*
	Female	*104,050 (48.8%)*	*174 (46.2%)*	*1 (ref)*
Residence Locality				
	Urban	*115,379 (54.1%)*	*225 (59.7%)*	*1.25 (1.02-1.53)*
	Rural	*97,398 (45.7%)*	*< 152 (< 40.3%)*	*1 (ref)*
	Unknown	*507 (0.2%)*	*< 6 (< 1.6%)*	*1.44 (0.20-10.31)*
Income Quintile				
	Q1 (lowest)	*55,960 (26.2%)*	*< 60 (< 15.9%)*	*0.54 (0.38-0.76)*
	Q2	*44,130 (20.7%)*	*84 (22.3%)*	*0.96 (0.70-1.32)*
	Q3	*39,717 (18.6%)*	*80 (21.2%)*	*1.02 (0.74-1.41)*
	Q4	*38,931 (18.3%)*	*86 (22.8%)*	*1.13 (0.82-1.56)*
	Q5 (highest)	*34,039 (16.0%)*	*67 (17.8%)*	*1 (ref)*
	Unknown	*507 (0.2%)*	*< 6 (< 1.6%)*	*1.14 (0.16-8.23)*
Gestation Period (Weeks)				
	<35	*5,005 (2.3%)*	*11 (2.9%)*	*1.25 (0.69-2.29)*
	35 to <37	*9,172 (4.3%)*	*20 (5.3%)*	*1.28 (0.81-2.02)*
	37 to <39	*43,729 (20.5%)*	*70 (18.6%)*	*0.94 (0.73-1.22)*
	39+	*155,378 (72.9%)*	*276 (73.2%)*	*1 (ref)*
Birth Weight (Grams)				
	<3000	*36,772 (17.2%)*	*61 (16.2%)*	*1.00 (0.53-1.91)*
	3000 to <3500	*70,402 (33.0%)*	*117 (31.0%)*	*1.00 (0.54-1.86)*

	3500 to <4500	*98,961 (46.4%)*	*188 (49.9%)*	*1.13 (0.62-2.08)*
	4500+	*6,560 (3.1%)*	*11 (2.9%)*	*1 (ref)*
	Unknown	*589 (0.3%)*	*0 (0.0%)*	*-*
Delivery Method				
	C- Section	*41,176 (19.3%)*	*72 (19.1%)*	*1.04 (0.80-1.34)*
	Vaginal	*172,108 (80.7%)*	*305 (80.9%)*	*1 (ref)*
Maternal Age				
	<20	*20,281 (9.5%)*	*27 (7.2%)*	*0.95 (0.61-1.49)*
	20-24	*46,525 (21.8%)*	*64 (17.0%)*	*1 (ref)*
	25-29	*63,573 (29.8%)*	*112 (29.7%)*	*1.29 (0.95-1.76)*
	30-34	*55,341 (25.9%)*	*133 (35.3%)*	*1.77 (1.32-2.39)*
	35+	*27,564 (12.9%)*	*41 (10.9%)*	*1.12 (0.76-1.66)*
Feeding Method (at birth)				
	Exclusive	*107,926 (50.6%)*	*221 (58.6%)*	*1 (ref)*
	Partially	*60,810 (28.5%)*	*81 (21.5%)*	*0.78 (0.60-1.00)*
	Formula Fed	*40,959 (19.2%)*	*67 (17.8%)*	*0.83 (0.63-1.09)*
	Unknown	*3,589 (1.7%)*	*8 (2.1%)*	*1.12 (0.56-2.27)*
Multiple Birth				
	No	*210,338 (98.6%)*	*> 371 (> 98.4%)*	*1 (ref)*
	Yes	*2,946 (1.4%)*	*< 6 (< 1.6%)*	*0.80 (0.30-2.15)*
Parity				
	0	*80,644 (37.8%)*	*167 (44.3%)*	*0.82 (0.75-0.90)*
	1	*69,156 (32.4%)*	*137 (36.3%)*	
	2	*33,655 (15.8%)*	*44 (11.7%)*	
	3	*14,577 (6.8%)*	*20 (5.3%)*	
	≥4	*14,472 (6.8%)*	*9 (2.4%)*	
	Unknown	*780 (0.4%)*	*0 (0.0%)*	
Number of Children in Household				
	1	*80,776 (37.9%)*	*163 (43.2%)*	*0.84 (0.77-0.92)*
	2	*71,271 (33.4%)*	*132 (35.0%)*	
	3	*34,179 (16.0%)*	*55 (14.6%)*	

	≥4	*26,816 (12.6%)*	*< 27 (< 7.2%)*	
	Unknown	*242 (0.1%)*	*< 6 (< 1.6%)*	
Maternal Pre-pregnancy Exposure				
	No	*138,252 (64.8%)*	*243 (64.5%)*	*1 (ref)*
	Yes	*75,032 (35.2%)*	*134 (35.5%)*	*1.01 (0.82-1.25)*
Maternal Post-pregnancy Exposure				
	No	*137,646 (64.5%)*	*254 (67.4%)*	*1 (ref)*
	Yes	*75,638 (35.5%)*	*123 (32.6%)*	*0.87 (0.70-1.07)*
Antenatal Exposure				
	No	*134,917 (63.3%)*	*222 (58.9%)*	*1 (ref)*
	Yes	*78,367 (36.7%)*	*155 (41.1%)*	*1.20 (0.98-1.47)*
Total Number of Courses				
	None	*134,917 (63.3%)*	*222 (58.9%)*	*1 (ref)*
	1	*47,181 (22.1%)*	*105 (27.9%)*	*1.35 (1.07-1.71)*
	2	*17,923 (8.4%)*	*31 (8.2%)*	*1.05 (0.72-1.53)*
	≥3	*13,263 (6.2%)*	*19 (5.0%)*	*0.85 (0.53-1.36)*
J01C				
	Unexposed	*160,793 (75.4%)*	*270 (71.6%)*	*1 (ref)*
	Exposed	*52,491 (24.6%)*	*107 (28.4%)*	*1.17 (0.94-1.46)*
J01D				
	Unexposed	*200,300 (93.9%)*	*363 (96.3%)*	*1 (ref)*
	Exposed	*12,984 (6.1%)*	*14 (3.7%)*	*0.69 (0.41-1.18)*
J01F				
	Unexposed	*198,196 (92.9%)*	*345 (91.5%)*	*1 (ref)*
	Exposed	*15,088 (7.1%)*	*32 (8.5%)*	*1.20 (0.84-1.72)*
J01A, J01M, J01X				
	Unexposed	*197,234 (92.5%)*	*353 (93.6%)*	*1 (ref)*
	Exposed	*16,050 (7.5%)*	*24 (6.4%)*	*0.88 (0.58-1.34)*
J01E				
	Unexposed	*207,678 (97.4%)*	*368 (97.6%)*	*1 (ref)*
	Exposed	*5,606 (2.6%)*	*9 (2.4%)*	*0.81 (0.42-1.57)*

Trimester 1				
	Unexposed	*178,783 (83.8%)*	*316 (83.8%)*	*1 (ref)*
	Exposed	*34,501 (16.2%)*	*61 (16.2%)*	*0.98 (0.74-1.30)*
Trimester 2				
	Unexposed	*174,020 (81.6%)*	*301 (79.8%)*	*1 (ref)*
	Exposed	*39,264 (18.4%)*	*76 (20.2%)*	*1.12 (0.86-1.45)*
Trimester 3				
	Unexposed	*182,014 (85.3%)*	*317 (84.1%)*	*1 (ref)*
	Exposed	*31,270 (14.7%)*	*60 (15.9%)*	*1.05 (0.79-1.39)*
0-3 Months				
	Unexposed	*202,944 (95.2%)*	*359 (95.2%)*	*1 (ref)*
	Exposed	*10,340 (4.8%)*	*18 (4.8%)*	*0.83 (0.51-1.34)*
3-6 Months				
	Unexposed	*181,972 (85.3%)*	*312 (82.8%)*	*1 (ref)*
	Exposed	*31,312 (14.7%)*	*65 (17.2%)*	*1.04 (0.78-1.37)*
6-12 Months				
	Unexposed	*128,409 (60.2%)*	*196 (52.0%)*	*1 (ref)*
	Exposed	*84,875 (39.8%)*	*181 (48.0%)*	*1.28 (1.03-1.58)*
Birth to 1 Year				
	Unexposed	*115,509 (54.2%)*	*173 (45.9%)*	*1 (ref)*
	Exposed	*97,775 (45.8%)*	*204 (54.1%)*	*1.26 (1.02-1.56)*
>1 Years				
	Unexposed	*34,035 (16.0%)*	*52 (13.8%)*	*1 (ref)*
	Exposed	*179,249 (84.0%)*	*325 (86.2%)*	*0.58 (0.43-0.79)*
Year of Birth				
	1996-1999	*50,739 (23.8%)*	*170 (45.1%)*	*1 (ref)*
	2000-2003	*47,684 (22.4%)*	*132 (35.0%)*	*0.95 (0.75-1.20)*
	2004-2007	*48,953 (23.0%)*	*57 (15.1%)*	*0.57(0.42-0.78)*
	2008-2012	*65,908 (30.9%)*	*18 (4.8%)*	*0.32 (0.19-0.53)*
Season of Birth				
	Winter	*49,604 (23.3%)*	*91 (24.1%)*	*1.12 (0.84-1.50)*

	Spring	*54,360 (25.5%)*	*111 (29.4%)*	*1.25 (0.95-1.65)*
	Summer	*56,111 (26.3%)*	*89 (23.6%)*	*1 (ref)*
	Fall	*53,209 (24.9%)*	*86 (22.8%)*	*1.05 (0.78-1.42)*
Child's Hospital Utilization				
	No	*171,586 (80.4%)*	*251 (66.6%)*	*1 (ref)*
	Yes	*41,698 (19.6%)*	*126 (33.4%)*	*5.91 (4.74-7.38)*
Child's Physician Utilization				
	<6	*57,311 (26.9%)*	*38 (10.1%)*	*1 (ref)*
	6 to 10	*53,943 (25.3%)*	*48 (12.7%)*	*1.50 (0.93-2.28)*
	11 to 18	*55,532 (26.0%)*	*112 (29.7%)*	*3.93 (2.72-5.67)*
	19+	*46,498 (21.8%)*	*179 (47.5%)*	*7.69 (5.42-10.92)*
Mother's Hospital Utilization				
	No	*109,787 (51.5%)*	*236 (62.6%)*	*1 (ref)*
	Yes	*103,497 (48.5%)*	*141 (37.4%)*	*0.74 (0.60-0.91)*
Mother's Physician Utilization				
	<6	*61,536 (28.9%)*	*132 (35.0%)*	*1 (ref)*
	6 to 12	*46,046 (21.6%)*	*89 (23.6%)*	*0.93 (0.71-1.21)*
	13 to 28	*54,265 (25.4%)*	*94 (24.9%)*	*0.91 (0.70-1.18)*
	29+	*51,437 (24.1%)*	*62 (16.4%)*	*0.75 (0.55-1.01)*

Table 9: Sensitivity Analysis (Outcome: Type 1 Diabetes Mellitus)

	Crude HR HR (95% CI)	*Adjusted for Covariates* HR (95% CI)*	*Adjusted for Covariates* + Infant Antibiotics HR (95% CI)*
Timing of antibiotic use			
Pre-Pregnancy	*1.01 (0.82-1.25)*	*1.01 (0.82-1.25)*	*1.02 (0.82-1.26)*
Antenatal	*1.20 (0.98-1.47)*	*1.21 (0.99-1.49)*	*1.22 (0.99-1.51)*
Postnatal	*0.87 (0.70-1.07)*	*0.87 (0.70-1.08)*	*0.87 (070-1.08)*
Number of antibiotic courses during pregnancy			
1	*1.35 (1.07-1.71)*	*1.35 (1.07-1.70)*	*1.36 (1.08-1.72)*
2	*1.05 (0.72-1.53)*	*1.07 (0.74-1.56)*	*1.08 (0.74-1.58)*
≥3	*0.85 (0.53-1.36)*	*0.89 (0.56-1.42)*	*0.90 (0.56-1.44)*
Timing of exposure in pregnancy			
Trimester 1	*0.98 (0.74-1.30)*	*0.98 (0.74-1.30)*	*0.99 (0.75-1.31)*
Trimester 2	*1.12 (0.86-1.45)*	*1.13 (0.88-1.47)*	*1.14 (0.88-1.48)*
Trimester 3	*1.05 (0.79-1.39)*	*1.07 (0.81-1.43)*	*1.08 (0.81-1.43)*
Type of antibiotics exposed			
J01C	*1.17 (0.94-1.46)*	*1.21 (0.96-1.52)*	*1.22 (0.97-1.53)*
J01D	*0.69 (0.41-1.18)*	*0.70 (0.41-1.21)*	*0.70 (0.41-1.21)*
J01F	*1.20 (0.84-1.72)*	*1.23 (0.85-1.77)*	*1.23 (0.85-1.77)*
J01(A, M, X)	*0.88 (0.58-1.34)*	*0.88 (0.58-1.34)*	*0.88 (0.58-1.34)*
J01E	*0.81 (0.42-1.57)*	*0.83 (0.43-1.61)*	*0.83 (0.43-1.62)*

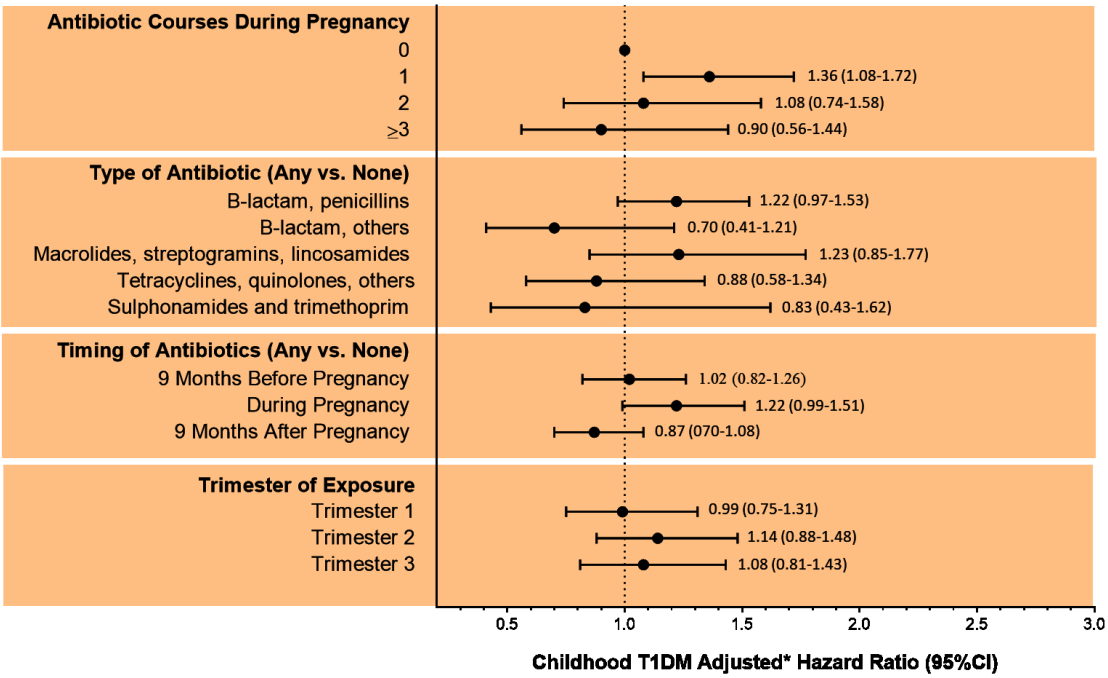


Fig. 3: Associations between maternal antibiotics during pregnancy and type 1 diabetes mellitus in their offspring by number of antibiotic courses, type of antibiotic, timing of exposure and trimester of exposure.

Adjust for confounders: maternal hyperglycemia, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

3.5. Type II Diabetes Mellitus

There were 215 children in the study population who developed T2DM, and among them, 97 had antenatal maternal antibiotic exposure (IR: 0.11 per 1,000 person-years; CI: 0.09-0.14). In comparison, children born to 118 non-exposed mothers developed T2DM (IR: 0.08 per 1,000 person-years; CI: 0.07-0.10). The risk of T2DM in exposed was higher than non-exposed with an IR of 1.41 (CI: 1.08-1.84).

Children born to mothers having any form of diabetes had a considerably higher event rate for T2DM (HR: 26.99 CI:20.24-35.99), indicating a very strong genetic influence. Male gender was associated with an approximate 50% risk-reduction for the development of T2DM in comparison to female gender which was used as a reference (HR: 0.48 CI:0.37-0.64). Children who were born into families in urban settings had a much lower even-rate (HR: 0.23 CI: 0.16-0.31) for T2DM than those born into families residing in rural areas. Similarly, children born into a family within the lowest income quintile (Q1) had a much higher event rate (HR: 8.34 CI: 4.49-15.86) in comparison to the children who were born into families with highest income quintile which was used as the reference. Concerning child birth at >39 weeks gestational age which was assigned HR of 1, the risk was significantly higher in all gestational age groups less than 39 weeks (Table 10).

In contrast, decreasing birth weight was associated with reduced event rates in children having birth weight <4500g. Compared to vaginally delivered children, those who were born through C-section had a much greater risk for childhood T2DM development (HR: 1.68 CI: 1.24-2.28). Compared to maternal age of 20-24 years at parturition, maternal age <20 years was associated with a greater risk for childhood T2M

in their offspring (HR: 1.21 CI: 0.83-1.77). However, children born to mothers greater than 24 years at parturition a significantly reduced risk for childhood T2DM development than the reference group. In comparison to children who were exclusively breastfed at birth, children who were only partially breastfed (HR: 3.08 CI: 2.20-4.33) or those who were formula fed (HR: 3.56 CI: 2.56-4.94) had a significantly higher risk for T2DM development. There were no children born as part of a multiple birth who went on to develop T2DM in the population. Children who had siblings and those children living in households having other children were at slightly higher risk of developing the condition in comparison to the eldest child and only child in the household respectively. Although the event rate was higher among children prescribed post-natal antibiotics between birth to 3 months as well as 3 to 6 months, the width of the confidence interval overlapped with the reference group (non-exposed), indicating non-significance. When considered together, children who were exposed to post-natal antibiotics did not have a significantly different event rate in comparison to non-exposed children. Children who were exposed to antibiotics beyond 1 year were found to have a significantly lower risk for T2DM that the non-exposed children, indicating a protective influence. Birth during any specific season was not found to alter the risk of T2DM development in the child. Similarly, both maternal hospital utilization (HR: 1.10 CI: 0.84-1.44) and the number of physician visits during pregnancy were not found to alter the incidence of disease in children.

Maternal antibiotic exposure during pre-pregnancy (HR: 1.04 CI: 0.79-1.37) and post-pregnancy (HR: 1.05 CI: 0.80-1.39) periods was not found to be associated with the disease development in their children. This lack of association was unchanged after adjustment for *a priori* covariates and infant antibiotics (Table 11). Although antenatal

maternal antibiotic exposure was associated with a marginally higher risk (HR: 1.39 CI: 1.06-1.82) of disease development in their children, the association was absent after adjusting for covariates (HR: 1.28 CI: 0.97-1.69). While maternal exposure to 1 or 2 courses of antibiotics during pregnancy was not associated with childhood T2DM, exposure to ≥3 courses of antibiotics during pregnancy was associated with higher T2DM even-rate (HR: 2.52 CI: 1.70-3.74). After adjusting for covariates and infant antibiotics, the risk associated with ≥3 courses of maternal antibiotics during pregnancy remained significantly high (HR: 1.93 CI: 1.28-2.93), indicating a possible dose-response relationship. Similarly, maternal antibiotic exposure during 3rd trimester was high, before (HR: 1.72 CI: 1.25-2.37) and after adjusting for covariates (HR: 1.62 CI: 1.17-2.24), indicating a possible temporal-relationship. Children born to mothers who were exposed to the antibiotic group comprising of tetracyclines, quinolones & other antibiotics during pregnancy had a higher event rate of T2DM (HR: 2.22 CI: 1.51-3.27). Although slightly reduced, the association was still evident after adjusting for *a priori* covariates and infant antibiotics (HR: 1.99 CI: 1.35-2.95). Antenatal exposure to none of the other antibiotic groups was found to alter the development of T2DM in children.

Table 10: Probable confounding influences in maternal antibiotic exposure and childhood type 2 diabetes mellitus

		Childhood Type 2 Diabetes Mellitus		
		No	***Yes***	***Crude Hazards Ratio (95% CI)***
		(N=213,446)	***(N=215)***	
Maternal Diabetes (any form)				
	No	*204,948 (96.0%)*	*146 (67.9%)*	*1 (ref)*
	Yes	*8,498 (4.0%)*	*69 (32.1%)*	*26.99 (20.24- 35.99)*
Gender				
	Male	*109,364 (51.2%)*	*73 (34.0%)*	*0.48 (0.37-0.64)*
	Female	*104,082 (48.8%)*	*142 (66.0%)*	*1 (ref)*
Residence Locality				
	Urban	*115,558 (54.1%)*	*46 (21.4%)*	*0.23 (0.16-0.31)*
	Rural	*97,380 (45.6%)*	*169 (78.6%)*	*1 (ref)*
	Unknown	*508 (0.2%)*	*0 (0.0%)*	*-*
Income Quintile				
	Q1 (lowest)	*55,884 (26.2%)*	*135 (62.8%)*	*8.34 (4.39-15.86)*
	Q2	*44,171 (20.7%)*	*43 (20.0%)*	*3.28 (1.65-6.52)*
	Q3	*39,787 (18.6%)*	*10 (4.7%)*	*0.85 (0.35-2.04)*
	Q4	*39,000 (18.3%)*	*17 (7.9%)*	*1.48 (0.68-3.24)*
	Q5 (highest)	*34,096 (16.0%)*	*10 (4.7%)*	*1 (ref)*
	Unknown	*508 (0.2%)*	*0 (0.0%)*	*-*
Gestation Period (Weeks)				
	<35	*5,006 (2.3%)*	*10 (4.7%)*	*3.28 (1.71-6.29)*
	35 to <37	*9,160 (4.3%)*	*32 (14.9%)*	*6.04 (4.06-9.00)*
	37 to <39	*43,725 (20.5%)*	*74 (34.4%)*	*2.92 (2.16-3.94)*
	39+	*155,555 (72.9%)*	*99 (46.0%)*	*1 (ref)*
Birth Weight (Grams)				
	<3000	*36,794 (17.2%)*	*39 (18.1%)*	*0.49 (0.27-0.90)*
	3000 to <3500	*70,458 (33.0%)*	*61 (28.4%)*	*0.40 (0.22-0.71)*

	3500 to <4500	*99,048 (46.4%)*	*101 (47.0%)*	*0.46 (0.27-0.81)*
	4500+	*6,557 (3.1%)*	*14 (6.5%)*	*1 (ref)*
	Unknown	*589 (0.3%)*	*0 (0.0%)*	*-*
Delivery Method				
	C- Section	*41,191 (19.3%)*	*57 (26.5%)*	*1.68 (1.24-2.28)*
	Vaginal	*172,255 (80.7%)*	*158 (73.5%)*	*1 (ref)*
Maternal Age				
	<20	*20,267 (9.5%)*	*41 (19.1%)*	*1.21 (0.83-1.77)*
	20-24	*46,514 (21.8%)*	*75 (34.9%)*	*1 (ref)*
	25-29	*63,634 (29.8%)*	*51 (23.7%)*	*0.50 (0.35-0.71)*
	30-34	*55,445 (26.0%)*	*29 (13.5%)*	*0.33 (0.22-0.51)*
	35+	*27,586 (12.9%)*	*19 (8.8%)*	*0.46 (0.28-0.75)*
Feeding Method (at birth)				
	Exclusive	*108,082 (50.6%)*	*< 66 (< 30.7%)*	*1 (ref)*
	Partially	*60,821 (28.5%)*	*70 (32.6%)*	*3.08 (2.20-4.33)*
	Formula Fed	*40,947 (19.2%)*	*79 (36.7%)*	*3.56 (2.56-4.94)*
	Unknown	*3,596 (1.7%)*	*< 6 (< 2.8%)*	*0.47 (0.07-3.37)*
Multiple Birth				
	No	*210,496 (98.6%)*	*215 (100.0%)*	*1 (ref)*
	Yes	*2,950 (1.4%)*	*0 (0.0%)*	*-*
Parity				
	0	*80,749 (37.8%)*	*62 (28.8%)*	
	1	*69,234 (32.4%)*	*59 (27.4%)*	*1.04 (1.02-1.06)*
	2	*33,657 (15.8%)*	*42 (19.5%)*	
	3	*14,574 (6.8%)*	*23 (10.7%)*	
	≥4	*14,452 (6.8%)*	*29 (13.5%)*	
	Unknown	*780 (0.4%)*	*0 (0.0%)*	
Number of Children in Household				
	1	*80,877 (37.9%)*	*62 (28.8%)*	*1.23 (1.12-1.34)*
	2	*71,340 (33.4%)*	*63 (29.3%)*	
	3	*34,190 (16.0%)*	*< 45 (< 20.9%)*	

	≥4	*26,797 (12.6%)*	*45 (20.9%)*	
	Unknown	*242 (0.1%)*	*< 6 (< 2.8%)*	
Maternal Pre-pregnancy Exposure				
	No	*138,358 (64.8%)*	*137 (63.7%)*	*1 (ref)*
	Yes	*75,088 (35.2%)*	*78 (36.3%)*	*1.04 (0.79-1.37)*
Maternal Post-pregnancy Exposure				
	No	*137,766 (64.5%)*	*134 (62.3%)*	*1 (ref)*
	Yes	*75,680 (35.5%)*	*81 (37.7%)*	*1.06 (0.80-1.39)*
Antenatal Exposure				
	No	*135,021 (63.3%)*	*118 (54.9%)*	*1 (ref)*
	Yes	*78,425 (36.7%)*	*97 (45.1%)*	*1.39 (1.06-1.82)*
Total Number of Courses				
	None	*135,021 (63.3%)*	*118 (54.9%)*	*1 (ref)*
	1	*47,242 (22.1%)*	*44 (20.5%)*	*1.06 (0.75-1.50)*
	2	*17,932 (8.4%)*	*22 (10.2%)*	*1.38 (0.88-2.18)*
	≥3	*13,251 (6.2%)*	*31 (14.4%)*	*2.52 (1.70-3.74)*
J01C				
	Unexposed	*160,913 (75.4%)*	*150 (69.8%)*	*1 (ref)*
	Exposed	*52,533 (24.6%)*	*65 (30.2%)*	*1.23 (0.92-1.64)*
J01D				
	Unexposed	*200,466 (93.9%)*	*197 (91.6%)*	*1 (ref)*
	Exposed	*12,980 (6.1%)*	*18 (8.4%)*	*1.90 (1.171-3.08)*
J01F				
	Unexposed	*198,347 (92.9%)*	*194 (90.2%)*	*1 (ref)*
	Exposed	*15,099 (7.1%)*	*21 (9.8%)*	*1.36 (0.87-2.14)*
J01A, J01M, J01X				
	Unexposed	*197,405 (92.5%)*	*182 (84.7%)*	*1 (ref)*
	Exposed	*16,041 (7.5%)*	*33 (15.3%)*	*2.46 (1.70-3.56)*
J01E				
	Unexposed	*207,842 (97.4%)*	*204 (94.9%)*	*1 (ref)*
	Exposed	*5,604 (2.6%)*	*11 (5.1%)*	*1.61 (0.88-2.96)*

Trimester 1				
	Unexposed	*178,933 (83.8%)*	*166 (77.2%)*	*1 (ref)*
	Exposed	*34,513 (16.2%)*	*49 (22.8%)*	*1.40 (1.01-1.94)*
Trimester 2				
	Unexposed	*174,153 (81.6%)*	*168 (78.1%)*	*1 (ref)*
	Exposed	*39,293 (18.4%)*	*47 (21.9%)*	*1.50 (0.75-1.47)*
Trimester 3				
	Unexposed	*182,170 (85.3%)*	*161 (74.9%)*	*1 (ref)*
	Exposed	*31,276 (14.7%)*	*54 (25.1%)*	*1.72 (1.25-2.37)*
0-3 Months				
	Unexposed	*203,107 (95.2%)*	*196 (91.2%)*	*1 (ref)*
	Exposed	*10,339 (4.8%)*	*19 (8.8%)*	*1.55 (0.96-2.53)*
3-6 Months				
	Unexposed	*182,116 (85.3%)*	*168 (78.1%)*	*1 (ref)*
	Exposed	*31,330 (14.7%)*	*47 (21.9%)*	*1.42 (1.01-2.00)*
6-12 Months				
	Unexposed	*128,475 (60.2%)*	*129 (60.0%)*	*1 (ref)*
	Exposed	*84,971 (39.8%)*	*86 (40.0%)*	*0.78 (0.58-1.05)*
Birth to 1 Year				
	Unexposed	*115,572 (54.1%)*	*109 (50.7%)*	*1 (ref)*
	Exposed	*97,874 (45.9%)*	*106 (49.3%)*	*0.98 (0.74-1.30)*
1+ Years				
	Unexposed	*34,022 (15.9%)*	*40 (18.6%)*	*1 (ref)*
	Exposed	*179,424 (84.1%)*	*175 (81.4%)*	*0.26 (0.18-0.38)*
Year of Birth				
	1996-1999	*50,765 (23.8%)*	*144 (67.0%)*	*1 (ref)*
	2000-2003	*47,749 (22.4%)*	*< 68 (< 31.6%)*	*0.77 (0.57-1.05)*
	2004-2007	*49,006 (23.0%)*	*< 6 (< 2.8%)*	*0.28 (0.10-0.78)*
	2008-2012	*65,926 (30.9%)*	*0 (0.0%)*	-
Season of Birth				
	Winter	*49,641 (23.3%)*	*54 (25.1%)*	*1.10 (0.75-1.61)*

	Spring	*54,412 (25.5%)*	*59 (27.4%)*	*1.09 (0.75-1.58)*
	Summer	*56,147 (26.3%)*	*53 (24.7%)*	*1 (ref)*
	Fall	*53,246 (24.9%)*	*49 (22.8%)*	*1.04 (0.70-1.53)*
Child's Hospital Utilization				
	No	*171,701 (80.4%)*	*155 (72.1%)*	*1 (ref)*
	Yes	*41,745 (19.6%)*	*60 (27.9%)*	*5.64 (4.18-7.60)*
Child's Physician Utilization				
	<6	*57,307 (26.8%)*	*46 (21.4%)*	*1 (ref)*
	6 to 10	*53,922 (25.3%)*	*57 (26.5%)*	*1.55 (1.05-2.28)*
	11 to 18	*55,554 (26.0%)*	*43 (20.0%)*	*1.37 (0.90-2.07)*
	19+	*46,663 (21.9%)*	*69 (32.1%)*	*2.52 (1.73-3.65)*
Mother's Hospital Utilization				
	No	*109,896 (51.5%)*	*127 (59.1%)*	*1 (ref)*
	Yes	*103,550 (48.5%)*	*88 (40.9%)*	*1.10 (0.84-1.44)*
Mother's Physician Utilization				
	<6	*61,577 (28.8%)*	*91 (42.3%)*	*1 (ref)*
	6 to 12	*46,084 (21.6%)*	*51 (23.7%)*	*0.81 (0.58-1.14)*
	13 to 28	*54,308 (25.4%)*	*51 (23.7%)*	*0.86 (0.61-1.21)*
	29+	*51,477 (24.1%)*	*22 (10.2%)*	*0.63 (0.40-1.01)*

Table 11: Sensitivity Analysis (Outcome: Type 2 Diabetes Mellitus)

	Crude HR HR (95% CI)	*Adjusted for Covariates* HR (95% CI)*	*Adjusted for Covariates* + Infant Antibiotics HR (95% CI)*
Timing of antibiotic use			
Pre-Pregnancy	*1.04 (0.79-1.37)*	*0.96 (0.73-1.27)*	*1.05 (0.79-1.40)*
Antenatal	*1.39 (1.06-1.82)*	*1.16 (0.88-1.52)*	*1.28 (0.97-1.69)*
Postnatal	*1.05 (0.80-1.39)*	*0.93 (0.71-1.23)*	*1.02 (0.77-1.36)*
Number of antibiotic courses during pregnancy			
1	*1.06 (0.75-1.50)*	*0.96 (0.68-1.36)*	*1.04 (0.74-1.48)*
2	*1.38 (0.88-2.18)*	*1.18 (0.75-1.85)*	*1.33 (0.84-2.12)*
≥3	*2.52 (1.70-3.74)*	*1.64 (1.10-2.46)*	*1.93 (1.28-2.93)*
Timing of exposure in pregnancy			
Trimester 1	*1.40 (1.01-1.94)*	*1.22 (0.88-1.71)*	*1.32 (0.94-1.84)*
Trimester 2	*1.05 (0.75-1.47)*	*0.87 (0.62-1.22)*	*0.93 (0.66-1.31)*
Trimester 3	*1.72 (1.25-2.37)*	*1.53 (1.11-2.11)*	*1.62 (1.17-2.24)*
Type of antibiotics exposed			
J01C	*1.10 (0.81-1.47)*	*1.01 (0.75-1.36)*	*1.11 (0.82-1.50)*
J01D	*1.57 (0.96-2.58)*	*1.03 (0.62-1.72)*	*1.07 (0.64-1.78)*
J01F	*1.20 (0.76-1.89)*	*1.12 (0.70-1.79)*	*1.21 (0.76-1.94)*
J01(A, M, X)	*2.22 (1.51-3.27)*	*1.98 (1.34-2.92)*	*1.99 (1.35-2.95)*
J01E	*1.22 (0.66-2.27)*	*0.90 (0.47-1.73)*	*0.97 (0.51-1.87)*

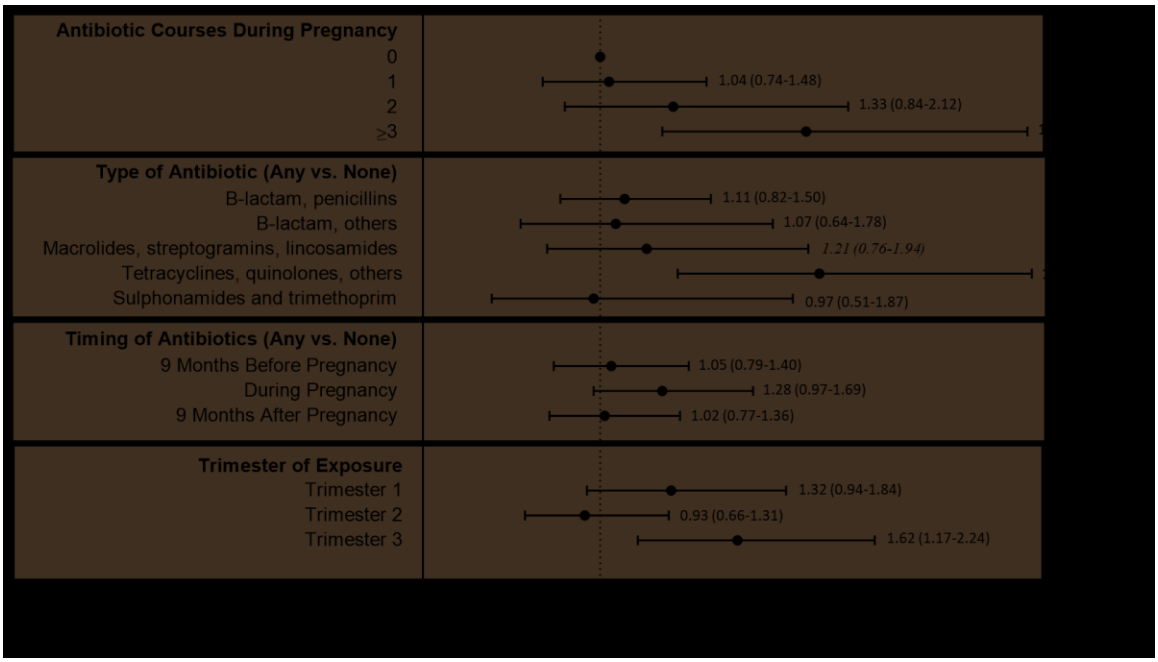


Fig. 4: Associations between maternal antibiotics during pregnancy and type 2 diabetes mellitus in their offspring by number of antibiotic courses, type of antibiotic, timing of exposure and trimester of exposure.

Adjust for confounders: maternal hyperglycemia, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

3.6. Cholelithiasis

There were 433 cases, comprising 0.2% of the total enrolled children, who developed cholelithiasis. Children whose mothers were exposed to antibiotics during pregnancy (IR: 0.16 per 1,000 person-years; CI: 0.14-0.19) had greater risk for cholelithiasis than children whose mothers did not receive antibiotics during pregnancy (IR: 0.23 per 1,000 person-years; CI: 0.20-0.26) with an IRR of 1.38 (CI: 1.14-1.67).

Children who were born to mothers having cholelithiasis had a significantly higher event rate for the condition in comparison to children born to cholelithiasis-naïve mothers (HR: 1.91 CI: 1.29-2.84). Compared to female gender, which was used as the reference, male gender was found to have a protective influence (HR: 0.31 CI: 0.25-0.39). Similarly, children born into families residing in urban localities were found to have a significantly reduced risk for cholelithiasis (HR: 0.44 CI: 0.36-0.54) compared to children residing in rural localities. Concerning children born in higher income families (Table 12) those who were born to the lower income families had an increasing risk, with the greatest risk noticed among children born to families with lowest income quintile (HR: 4.33 CI: 2.97-6.30). Although gestational age less than 35 weeks was associated with a higher event rate (HR: 1.55 CI: 0.92-2.60), the difference was non-significant concerning the reference (≥39-week gestational age). The child's birth weight was not found to have a considerable influence on the disease outcome. Children born through C-section had no significant difference in risk (HR: 0.89 CI: 0.69-1.14) compared to children born through vaginal delivery. After stratifying for maternal age at parturition, maternal age of >20 years was found to be associated with a higher risk of cholelithiasis in children (HR: 1.48 CI: 1.13-1.93), compared to the reference maternal age of 20-24 years. Children who were born to mothers

in age group>24 years at parturition was found to have around 50% reduction in the risk of cholelithiasis. Children who were formula fed at birth progressed to develop cholelithiasis at a rate more than twice as fast as exclusively breastfed children (HR: 2.33 CI: 1.88-2.89). The event rate in partially breastfed children was only marginally significant (HR: 1.31 CI: 1.01-1.69). There were 427 children born from singleton pregnancies and 6 children from multiple births who developed childhood cholelithiasis (HR: 0.73 CI: 0.27-1.96). Although parity ≥1 was not associated with an increased event rate of cholelithiasis, children who were born into households having other children had an unremarkably higher event rate (HR: 1.11 CI: 1.04-1.19). Similar unremarkable elevation in risk of childhood cholelithiasis was present in children who were prescribed antibiotics during postnatal period of 3 months from birth (HR: 1.47 CI: 1.03-2.10) and 3-6 months (HR: 1.37 CI: 1.07-1.75). Childhood prescription of antibiotics beyond the age of 1 year was associated with a risk reduction of around 75% (HR:0.25 CI: 0.20-0.32). Season of birth was not associated with an alteration in the event rate of childhood cholelithiasis. Interestingly, though maternal hospital utilization during pregnancy was not associated with an altered risk of cholelithiasis, higher physician consultation was associated with a reduction in the event rate.

Maternal antibiotic exposure during pre-pregnancy period was associated with a slightly increased risk for cholelithiasis (HR: 1.14 CI: 1.01-1.49). A marginally higher risk was observed among children born to mothers who had exposure to antibiotics during pregnancy (HR: 1.36 CI: 1.13-1.65) and up to 9-months after parturition (HR: 1.34 CI: 1.11-1.63). After adjusting for covariates, the event rate was found to be similar irrespective of the timing of antibiotic use (Table 13). Post-natal period was associated

with an aHR of 1.46 (CI: 1.21-1.77), while pre-pregnancy and post-pregnancy periods were associated with aHR of 1.40 (CI: 1.15-1.70) and 1.52 (CI: 1.25-1.84) respectively. Children born to mothers who were prescribed three or more courses of antibiotic (aHR: 2.23 CI: 1.64-3.03) have a higher event rate of cholelithiasis than children born to mothers prescribed one (aHR: 1.23 CI: 0.97-1.56) or two (aHR: 1.51 CI: 1.10-2.09) courses. Though maternal antibiotic exposure during trimester 1 and trimester 3 were not associated with cholelithiasis development in their children, exposure during trimester 2 was found to significantly increase the event rate both before (aHR: 1.64 CI: 1.31-2.03) and after adjusting for covariates (aHR: 1.73 CI: 1.39-2.16). Among the antibiotics prescribed during pregnancy, the group comprising of macrolides, streptogramins and lincosamides were associated with an increased event rate (aHR:1.54 CI:1.13-2.10). The risk was unremarkably higher for the group comprising of beta-lactams & penicillins (aHR: 1.34 CI: 1.09-1.65) and group comprising sulphonamides and trimethoprim (aHR: 1.67 CI: 1.09-2.54).

Table 12: Probable confounding influences in maternal antibiotic exposure and childhood cholelithiasis

		Childhood Cholelithiasis		
		No	***Yes***	***Crude Hazards Ratio (95% CI)***
		(N=213,228)	***(N=433)***	
Mother has Condition				
	No	204,133 (95.7%)	407 (94.0%)	*1 (ref)*
	Yes	9,095 (4.3%)	26 (6.0%)	*1.91 (1.29-2.84)*
Gender				
	Male	109,329 (51.3%)	108 (24.9%)	*0.31 (0.25-0.39)*
	Female	103,899 (48.7%)	325 (75.1%)	*1 (ref)*
Residence Locality				
	Urban	115,454 (54.1%)	< 151 (< 34.9%)	*0.44 (0.36-0.54)*
	Rural	97,267 (45.6%)	282 (65.1%)	*1 (ref)*
	Unknown	507 (0.2%)	< 6 (< 1.4%)	*0.75 (0.11-5.34)*
Income Quintile				
	Q1 (lowest)	55,802 (26.2%)	217 (50.1%)	*4.33 (2.97-6.30)*
	Q2	44,129 (20.7%)	85 (19.6%)	*2.09 (1.39-3.16)*
	Q3	39,747 (18.6%)	50 (11.5%)	*1.37 (0.88-2.15)*
	Q4	38,968 (18.3%)	49 (11.3%)	*1.38 (0.88-2.16)*
	Q5 (highest)	34,075 (16.0%)	< 32 (< 7.4%)	*1 (ref)*
	Unknown	507 (0.2%)	< 6 (< 1.4%)	*2.41 (0.33-17.63)*
Gestation Period (Weeks)				
	<35	5,001 (2.3%)	15 (3.5%)	*1.55 (0.92-2.60)*
	35 to <37	9,180 (4.3%)	12 (2.8%)	*0.70 (0.40-1.25)*
	37 to <39	43,706 (20.5%)	93 (21.5%)	*1.14 (0.90-1.43)*
	≥39	155,341 (72.9%)	313 (72.3%)	*1 (ref)*
Birth Weight (Grams)				
	<3000	36,766 (17.2%)	67 (15.5%)	*1.05 (0.56-1.99)*
	3000 to <3500	70,357 (33.0%)	162 (37.4%)	*1.33 (0.72-2.44)*

	3500 to <4500	98,956 (46.4%)	193 (44.6%)	*1.12 (0.61-2.05)*
	4500+	6,560 (3.1%)	11 (2.5%)	*1 (ref)*
	Unknown	589 (0.3%)	0 (0.0%)	-
Delivery Method				
	C- Section	41,178 (19.3%)	70 (16.2%)	*0.89 (0.69-1.14)*
	Vaginal	172,050 (80.7%)	363 (83.8%)	*1 (ref)*
Maternal Age				
	<20	20,218 (9.5%)	90 (20.8%)	*1.48 (1.13-1.93)*
	20-24	46,454 (21.8%)	135 (31.2%)	*1 (ref)*
	25-29	63,593 (29.8%)	92 (21.2%)	*0.50 (0.38-0.65)*
	30-34	55,391 (26.0%)	83 (19.2%)	*0.52 (0.40-0.69)*
	35+	27,572 (12.9%)	33 (7.6%)	*0.44 (0.30-0.64)*
Feeding Method (at birth)				
	Exclusive	107,961 (50.6%)	186 (43.0%)	*1 (ref)*
	Partially	60,804 (28.5%)	87 (20.1%)	*1.31 (1.01-1.69)*
	Formula Fed	40,877 (19.2%)	149 (34.4%)	*2.33 (1.88-2.89)*
	Unknown	3,586 (1.7%)	11 (2.5%)	*1.75 (0.95-3.21)*
Multiple Birth				
	No	210,282 (98.6%)	> 427 (> 98.6%)	*1 (ref)*
	Yes	2,946 (1.4%)	< 6 (< 1.4%)	*0.73 (0.27-1.96)*
Parity				
	0	80,660 (37.8%)	151 (34.9%)	*1.02 (1.00-1.05)*
	1	69,139 (32.4%)	154 (35.6%)	
	2	33,637 (15.8%)	62 (14.3%)	
	3	14,570 (6.8%)	27 (6.2%)	
	≥4	14,442 (6.8%)	39 (9.0%)	
	Unknown	780 (0.4%)	0 (0.0%)	
Number of Children in Household				
	1	80,797 (37.9%)	142 (32.8%)	*1.11 (1.04-1.19)*
	2	71,247 (33.4%)	156 (36.0%)	
	3	34,165 (16.0%)	69 (15.9%)	

	≥4	26,776 (12.6%)	66 (15.2%)	
	Unknown	243 (0.1%)	0 (0.0%)	
Maternal Pre-pregnancy Exposure				
	No	138,235 (64.8%)	260 (60.0%)	*1 (ref)*
	Yes	74,993 (35.2%)	173 (40.0%)	*1.23 (1.01-1.49)*
Maternal Post-pregnancy Exposure				
	No	137,655 (64.6%)	245 (56.6%)	*1 (ref)*
	Yes	75,573 (35.4%)	188 (43.4%)	*1.34 (1.11-1.63)*
Antenatal Exposure				
	No	134,899 (63.3%)	240 (55.4%)	*1 (ref)*
	Yes	78,329 (36.7%)	193 (44.6%)	*1.36 (1.13-1.65)*
Total Number of Courses				
	None	134,899 (63.3%)	240 (55.4%)	*1 (ref)*
	1	47,190 (22.1%)	96 (22.2%)	*1.14 (0.90-1.44)*
	2	17,909 (8.4%)	45 (10.4%)	*1.40 (1.01-1.91)*
	≥3	13,230 (6.2%)	52 (12.0%)	*2.10 (1.56-2.83)*
J01C				
	Unexposed	160,766 (75.4%)	297 (68.6%)	*1 (ref)*
	Exposed	52,462 (24.6%)	136 (31.4%)	*1.32 (1.07-1.61)*
J01D				
	Unexposed	200,262 (93.9%)	401 (92.6%)	*1 (ref)*
	Exposed	12,966 (6.1%)	32 (7.4%)	*1.57 (1.09-2.25)*
J01F				
	Unexposed	198,154 (92.9%)	387 (89.4%)	*1 (ref)*
	Exposed	15,074 (7.1%)	46 (10.6%)	*1.50 (1.10-2.03)*
J01A, J01M, J01X				
	Unexposed	197,188 (92.5%)	399 (92.1%)	*1 (ref)*
	Exposed	16,040 (7.5%)	34 (7.9%)	*1.14 (0.80-1.61)*
J01E				
	Unexposed	207,637 (97.4%)	409 (94.5%)	*1 (ref)*
	Exposed	5,591 (2.6%)	24 (5.5%)	*1.81 (1.20-2.73)*

Trimester 1				
	Unexposed	178,746 (83.8%)	353 (81.5%)	*1 (ref)*
	Exposed	34,482 (16.2%)	80 (18.5%)	*1.05 (0.82-1.34)*
Trimester 2				
	Unexposed	174,007 (81.6%)	314 (72.5%)	*1 (ref)*
	Exposed	39,221 (18.4%)	119 (27.5%)	*1.64 (1.31-2.03)*
Trimester 3				
	Unexposed	181,979 (85.3%)	352 (81.3%)	*1 (ref)*
	Exposed	31,249 (14.7%)	81 (18.7%)	*1.13 (0.88-1.45)*
0-3 Months				
	Unexposed	202,909 (95.2%)	398 (91.9%)	*1 (ref)*
	Exposed	10,319 (4.8%)	35 (8.1%)	*1.47 (1.03-2.10)*
3-6 Months				
	Unexposed	181,950 (85.3%)	345 (79.7%)	*1 (ref)*
	Exposed	31,278 (14.7%)	88 (20.3%)	*1.37 (1.07-1.75)*
6-12 Months				
	Unexposed	128,363 (60.2%)	264 (61.0%)	*1 (ref)*
	Exposed	84,865 (39.8%)	169 (39.0%)	*0.82 (0.67-1.02)*
Birth to 1 Year				
	Unexposed	115,482 (54.2%)	226 (52.2%)	*1 (ref)*
	Exposed	97,746 (45.8%)	207 (47.8%)	*1.01 (0.83-1.23)*
1+ Years				
	Unexposed	34,009 (15.9%)	101 (23.3%)	*1 (ref)*
	Exposed	179,219 (84.1%)	332 (76.7%)	*0.25 (0.20-0.32)*
Year of Birth				
	1996-1999	50,597 (23.7%)	312 (72.1%)	*1 (ref)*
	2000-2003	47,741 (22.4%)	75 (17.3%)	*0.50 (0.38-0.65)*
	2004-2007	48,982 (23.0%)	28 (6.5%)	*0.44 (0.29-0.67)*
	2008-2012	65,908 (30.9%)	18 (4.2%)	*0.33 (0.20-0.56)*
Season of Birth				
	Winter	49,584 (23.3%)	111 (25.6%)	*1.08 (0.83-1.41)*

	Spring	54,354 (25.5%)	117 (27.0%)	*1.04 (0.80-1.35)*
	Summer	56,089 (26.3%)	111 (25.6%)	*1 (ref)*
	Fall	53,201 (25.0%)	94 (21.7%)	*0.94 (0.71-1.24)*
Child's Hospital Utilization				
	No	171,635 (80.5%)	238 (55.0%)	*1 (ref)*
	Yes	41,593 (19.5%)	195 (45.0%)	*8.94 (7.35-10.86)*
Child's Physician Utilization				
	<6	57,253 (26.9%)	84 (19.4%)	*1 (ref)*
	6 to 10	53,861 (25.3%)	85 (19.6%)	*1.21 (0.90-1.63)*
	11 to 18	55,517 (26.0%)	99 (22.9%)	*1.55 (1.16-2.08)*
	19+	46,597 (21.9%)	165 (38.1%)	*2.89 (2.22-3.76)*
Mother's Hospital Utilization				
	No	109,754 (51.5%)	269 (62.1%)	*1 (ref)*
	Yes	103,474 (48.5%)	164 (37.9%)	*0.98 (0.80-1.19)*
Mother's Physician Utilization				
	<6	61,479 (28.8%)	189 (43.6%)	*1 (ref)*
	6 to 12	46,024 (21.6%)	111 (25.6%)	*0.88 (0.69-1.11)*
	13 to 28	54,277 (25.5%)	82 (18.9%)	*0.69 (0.53-0.90)*
	29+	51,448 (24.1%)	51 (11.8%)	*0.68 (0.49-0.93)*

Table 13: Sensitivity Analysis (Outcome: Cholelithiasis)

	Crude HR HR (95% CI)	*Adjusted for Covariates* HR (95% CI)*	*Adjusted for Covariates* + Infant Antibiotics HR (95% CI)*
Timing of antibiotic use			
Pre-Pregnancy	1.23 (1.01-1.49)	*1.24 (1.02-1.50)*	*1.40 (1.15-1.70)*
Antenatal	1.36 (1.13-1.65)	*1.30 (1.08-1.58)*	*1.46 (1.21-1.77)*
Postnatal	1.34 (1.11-1.63)	*1.35 (1.11-1.63)*	*1.52 (1.25-1.84)*
Number of antibiotic courses during pregnancy			
1	1.14 (0.90-1.44)	*1.12 (0.88-1.42)*	*1.23 (0.97-1.56)*
2	1.40 (1.01-1.91)	*1.31 (0.95-1.81)*	*1.51 (1.10-2.09)*
≥3	2.10 (1.56-2.83)	*1.86 (1.38-2.52)*	*2.23 (1.64-3.03)*
Timing of exposure in pregnancy			
Trimester 1	1.05 (0.82-1.34)	*1.04 (0.81-1.33)*	*1.12 (0.87-1.44)*
Trimester 2	1.64 (1.31-2.03)	*1.60 (1.28-1.99)*	*1.73 (1.39-2.16)*
Trimester 3	1.13 (0.88-1.45)	*1.02 (0.80-1.31)*	*1.09 (0.85-1.40)*
Type of antibiotics exposed			
J01C	1.32 (1.07-1.61)	*1.20 (0.98-1.48)*	*1.34 (1.09-1.65)*
J01D	1.57 (1.09-2.25)	*1.19 (0.82-1.72)*	*1.23 (0.85-1.78)*
J01F	1.50 (1.10-2.03)	*1.43 (1.05-1.94)*	*1.54 (1.13-2.10)*
J01(A, M, X)	1.14 (0.80-1.61)	*0.98 (0.69-1.41)*	*1.01 (0.72-1.45)*
J01E	1.81 (1.20-2.73)	*1.57 (1.03-2.39)*	*1.67 (1.09-2.54)*

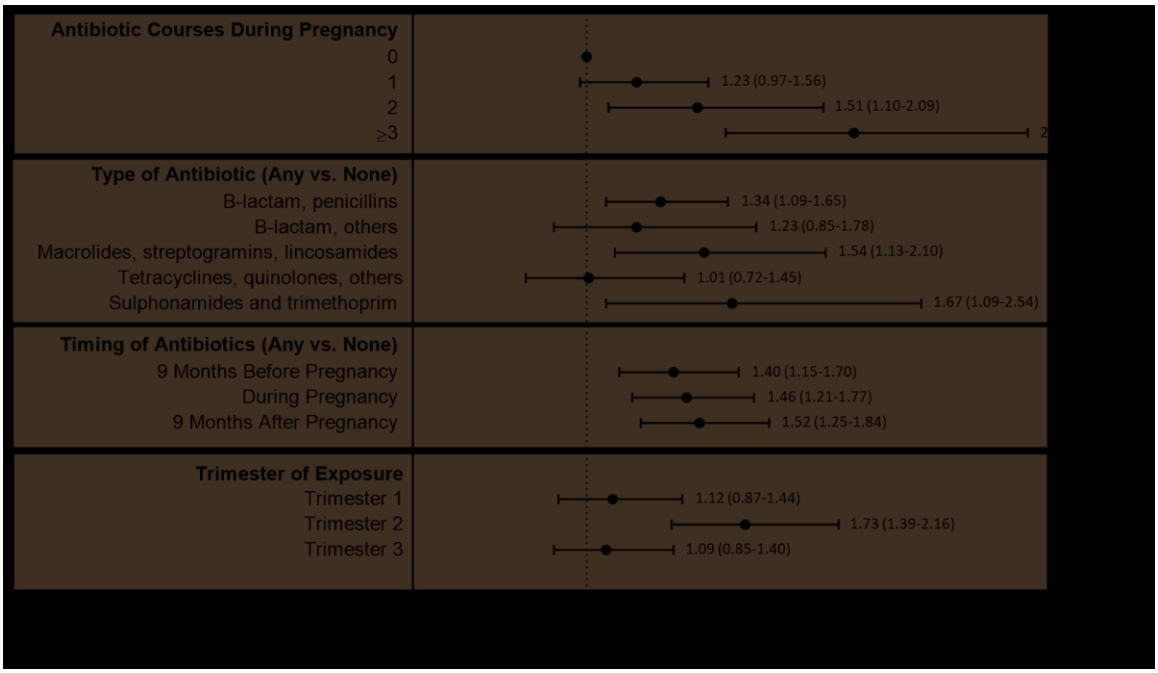
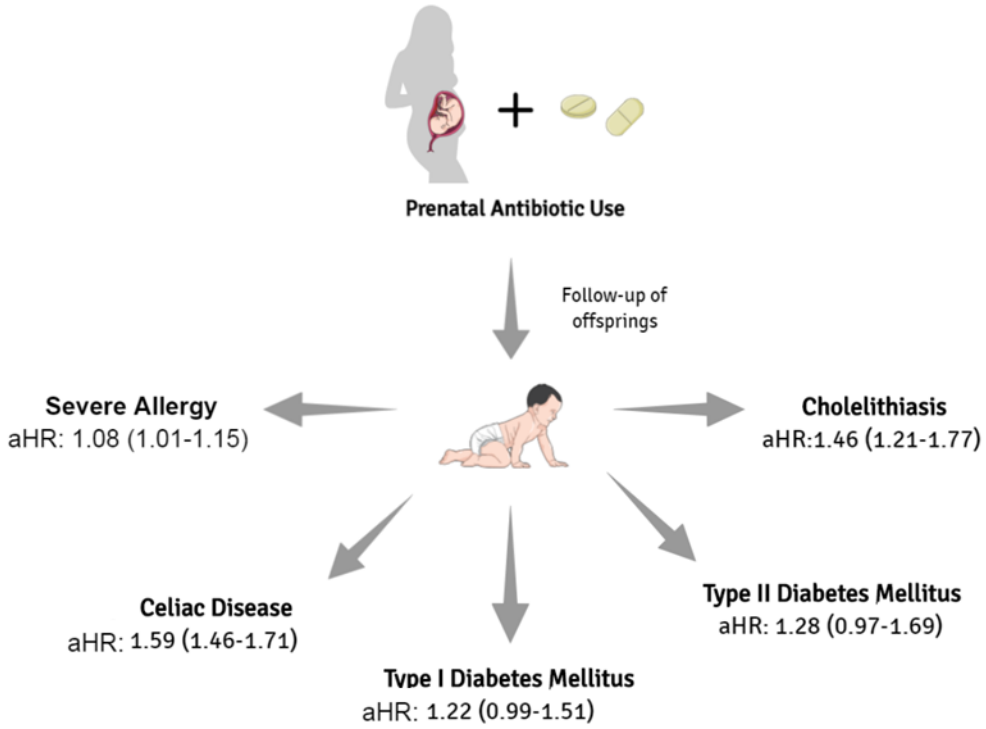


Fig. 5: Associations between maternal antibiotics during pregnancy and cholelithiasis in their offspring by number of antibiotic courses, type of antibiotic, timing of exposure and trimester of exposure.
Adjust for confounders: maternal cholelithiasis, infant sex, the location of residence, the length of gestation, and the number of children in a household. Infant antibiotics = any antibiotics in the first year of life.

Figure 1: Summary of Results



Chapter 4. DISCUSSION

The retrospective cohort study aimed at identifying the impact of maternal antibiotics during pregnancy and development of five chronic diseases during childhood found that more than one-third of the pregnant women in the province of Manitoba were exposed to antibiotics. Although there is wide variation in the literature regarding the proportion of maternal antibiotic use during pregnancy, the values usually range between 20-40% [166, 194, 195]. Antenatal antibiotic use was marginally higher among mothers from rural families and mothers from least affluent households, which could be due to the improved health literacy among women from urban and affluent families. As expected, the most commonly prescribed group of antibiotics were β-lactam antibacterials, penicillins (J01C) since they comprise one of the safest antibiotic options during pregnancy. The use of antibiotics during each trimester was higher than the data reported from most European Union countries but slightly lower than the results of a study from the USA [195, 196].

4.1. Severe Allergy

Our study, comprising of data collected from provincial registries, did not find a clear association between antenatal antibiotic exposure and severe allergy in the progeny. Considering our data size, there was only a marginal increase in the risk of severe allergy, irrespective of whether maternal exposure to antibiotics was before, during or after pregnancy. Most risk of confounding bias was eliminated by adjusting the model for numerous *a priori* covariates. The rationale for the study was the evidences indicating that the pioneer microbiota of infants has a maternal signature, whose alteration by antibiotics during pregnancy could predispose the offspring to develop childhood chronic diseases

[197]. Although not all human studies found an association, the majority of the published literature was supportive of the hypothesis that antibiotic consumption by mothers during pregnancy increases the risk of allergy development in their offspring [198]. To our knowledge, there have been no published studies on maternal antibiotic exposure and development of childhood severe allergy.

Contrasting with maternal exposure, infant antibiotic exposure during the first year of life (unadjusted) was associated with a higher risk in our study and our results were similar to the observations by Mitre et al. (aHR, 1.51, 95% CI 1.38-1.66) [199]. In the absence of comparable studies on severe allergy, we compared our findings with studies evaluating the development of non-severe allergic reactions. There was a mild increase in the risk of severe allergy in children who had parental exposure to >2 courses of antibiotics. McKeever et al. also found a dose-response relation for asthma (aHR: 1.68; 95% CI 1.51-1.87), eczema (aHR: 1.17; 95% CI 1.06-1.29) and hay fever (aHR: 1.56; 95% CI 1.22-2.01) in their UK birth cohort of 24,690 children [200]. Our study did not find an increased association of severe allergy with any specific trimester as opposed to increased risk during 2nd and 3rd trimester (OR of 1.18; 95% CI: 1.04-2.94) observed by Jedrychowski et al. [198]. The observation is in contradiction of the hypothesis that the antibiotic exposure during the third trimester is most crucial, considering the possibilities of transfer to the offspring during delivery. McKeever et al. and Metsälä J et al. found an increased risk of allergy in children irrespective of the class of antibiotic consumed by the mother during pregnancy [200, 201]. As opposed to their findings, we observed a marginally increased risk of severe allergy in Manitoban children whose mothers were exposed to the class of macrolides, streptogramins and lincosamides during pregnancy.

Stokholm proposed that the antenatal exposure may not have a direct causative influence on the disease outcome, but possibly confounded by the inherited susceptibility to infections [190]. Uniformly increased association between exposure and outcome across the nine months around pregnancy in our study conform to their hypothesis. The long-lasting impact of antibiotics on maternal microbiota can be considered the alternative explanation [202–204]. The microbiota disruption induced during antenatal period might persist beyond the duration of pregnancy and might be horizontally transferred to the infant through breast milk. Also, there is a possibility that the dysbiosis associated with antibiotic consumption before pregnancy might persist and be transferred to the fetus [191]. Highly lipophilic drugs such as quinolones and macrolides can accumulate in tissues at a relatively higher concentration than in plasma and use of such antibiotics during pregnancies or even pre-pregnancy period could create repositories, which have a long-lasting impact on maternal and infant microbiota [178]. Inherited susceptibility to infection in mothers predisposes continuous antibiotic use irrespective of whether it is before, during or after pregnancy. A repeated insult to the microbiome by broad-spectrum antibiotics could prolong the duration of dysbiosis as was evident from an increased association for more than two courses of antibiotics. Even though there is a likelihood that mothers who have an increased physician seeking behaviour could lead to increased diagnosis of atopy in their children, diagnostic classification as severe allergy requires a more intense reaction than for non-severe allergy.

In this population-based study, although we perceived a weak dose-response relationship between antenatal antibiotics and manifestation of severe childhood allergy, the risk was uniformly elevated with exposures in pre-pregnancy as well as post-pregnancy setting. The

perturbation on microbiota (which is the hypothesized mechanism through which antibiotic use leads to disease development) would be much stronger during the last trimester due to both vertical and horizontal transfer than the impact associated with antibiotic consumed during the first trimester. The absence of an increased event rate among children whose mothers had antibiotic exposure during the third trimester questions the possibility of an association between maternal antibiotics and severe childhood allergy.

4.2. Celiac Disease

Celiac disease is an autoimmune disease of the small intestine characterized by villous atrophy in genetically susceptible individuals. The most significant risk factor among the covariates analyzed was maternal celiac disease, which conforms to the increased risk observed among genetically predisposed individuals. Unlike former studies, which found higher risk among female children, our findings did not indicate a gender-specific risk for celiac disease development [205]. The higher risk among children born to families in urban residence in comparison to rural residence might be indicative of a more diverse environmental microbiota in the latter. The urban residents have reduced exposure to environmental microbes and this deficiency might be reflected in the inadequate development of their immune system. The protective influence of rural birth is further evident from the finding that children who are born into households with other children have a marginally reduced risk. Presence of other children in the household increases the chance of childhood exposure to more diverse microbes that helps in the development of their microbiota and thereby the immune system as per the OF hypothesis (the hygiene hypothesis alternatively explains the same regarding a higher infectious load in a house with siblings). The marginally higher event rate among prematurely born children could be

due to admission to the intensive care and prolonged separation from mothers compared to term-infants, which affect the pioneer flora and thereby immune system development. Although multiple studies have considered maternal celiac disease as a risk for pregnancy complications including premature delivery and low birth weight, we did not observe an increased risk among low-birth-weight infants [206, 207]. Namatovu et al. observed that maternal age higher than 35 years imparted a protective effect on childhood celiac disease [208]. They hypothesized that this could be due to the prolonged breast-feeding practices among older mothers. However, our findings did not indicate an increase/decrease in the risk of celiac disease in any specific maternal age group. Except for a Finnish study, most published literature indicated a seasonal influence on the development of Celiac disease with people born in summer or spring having a higher risk [209–211]. The seasonal patterns of viral illness and vitamin D levels are both hypothesized as possible reasons. Our study did not find any seasonal association, which could be due to the difference in seasonal durations in Manitoba compared to other places. Our findings of higher event rate among children born through C-section comply with findings from numerous studies, which supported an increased risk of chronic inflammatory disorders among children born through C-section [212–215].

Although the findings from our study indicated a possible association between antenatal maternal antibiotic use and development of childhood celiac disease in their children, the exclusivity of the association is questionable due to similarly higher event rate among children born to mothers who were exposed to antibiotics in the pre-pregnancy and postpartum setting. In a similar study evaluating the association of maternal antibiotics and childhood asthma, Stokholm et al. found similar association irrespective of the timing of

exposure and inferred the possibility of a confounding influence of maternal infection susceptibility rather than antenatal antibiotics [190]. However, our results of higher event rate among children born to mothers exposed to antibiotics during pregnancy were in contradiction to the findings from a Swedish cohort (ABIS) cohort, which showed a lack of association [174]. The lack of association could possibly be due to a much smaller sample size of celiac disease patients in the cohort (n=46). Although we did not find a temporal relationship concerning trimester of exposure, there was an evident dose-response relationship. Although this once again emphasizes the influence of maternal infection susceptibility, the event rate among children of mothers exposed to different groups of antibiotics is different. While penicillin use during pregnancy has a significant influence on the childhood celiac disease outcome, exposure to most other antibiotic groups (except the group comprising of macrolides, streptogramins and lincosamides) are only marginally significant indicating that certain antibiotic exposure, irrespective of the maternal infection susceptibility, does potentiate disease development in their offspring. Also, the event rate in offspring is similar, irrespective of the frequency of maternal physician consultation or maternal hospital utilization, which questions the magnitude of the confounding influence associated with maternal infection susceptibility (or physician-seeking behaviour). A Norwegian cohort study by Mårild et al. did not find a clear association between maternal infection during pregnancy and development of celiac disease in their offspring [216]. Overall, although our findings indicate that multiple-courses of antibiotics and exposure to penicillins during pregnancy might lead to a higher event rate, the ambiguity in the risk associated with maternal antibiotic exposure around the time of pregnancy makes this association uncertain.

4.3. Type I Diabetes Mellitus

The condition is characterized by autoimmune destruction of insulin producing β-cells in the pancreas. Although genetics plays a significant role in the disease development, the rapid increase in the incidence of T1DM among children in the past few decades indicate the role of environmental exposures and lifestyle as strong determinants of the disease outcome because genetic changes alone takes several generations to considerably alter disease incidence [217]. Although maternal type 1 diabetes during pregnancy is considered a determinant of childhood T1DM, paternal T1DM is considered to have a stronger influence [218]. Moreover, the predictive power of maternal gestational diabetes and other types of diabetes in the development of childhood type 1 diabetes is weaker than that of maternal T1DM. The lack of an association observed in our study between maternal diabetes and childhood T1DM might be due to the same reason. However, this finding contradicts the results of some previous studies which found an association between any form of maternal diabetes and childhood T1DM [219, 220]. Although sexual dimorphism has been reported for T1DM, with boys having slightly higher risk than females, our findings were nonsignificant for unclear reasons [221]. Urban residence, which is among the risk factors for several autoimmune conditions due to the reduced exposure to diverse environmental microorganisms, was found to impart a slightly marginal risk increase.

Similarly, children born into families with the lowest income quintile had risk-reduction compared to the highest income family. The available literature on the impact of socioeconomic circumstances and childhood T1DM have reported conflicting findings, which makes the interpretation of our results challenging [222–225]. Similar to the observations of Lindell et al., we did not find an association between gestational period

and disease development [220]. However, unlike their findings where parity did not influence the disease outcome, we observed a marginal risk-reduction associated with higher parity. Caesarean-section delivery is a known risk factor for alteration in the diversity of gut microbiota and thereby affecting the development of T1DM [214]. In our study population, there was no significant difference in the T1DM disease outcome among children born through C-section and vaginal delivery. Another major factor, which influences the infant microbiota, is the breastfeeding practice, where exclusive breastfeeding for longer duration is found to have a positive impact on the infant microbiota. Although our data lacked long-term follow-up on breastfeeding practices, recent findings from the CHILD pregnancy cohort indicated that the breastfeeding practices in the hospital setting is a good indicator of the long-term feeding practices [226]. Among our findings, we did not find an association between breastfeeding practices during hospital stay and childhood outcomes. We found that maternal age of 30-34 years at parturition was associated with an increased risk of T1DM in their offspring. A meta-analysis comprising of 5 cohort studies and 25 case-control studies found a positive association between maternal age and T1DM in children, with an estimated increase of 5% in the risk for each 5-year increase in maternal age [227]. However, the offspring of mothers who were 35 years or older at parturition did not have an increased risk for disease development in our population for unknown reasons. The protective effect of maternal hospital utilization during pregnancy might be due to better glycemic control associated with timely hospital visits.

The impact of antibiotic exposure either directly (childhood antibiotic use) or indirectly (maternal antibiotics) was not associated with an alteration in the event rate of

type 1 diabetes. Also, lack of a temporal gradient and dose-response relationship indicate the absence of any association between maternal antibiotics and the development of T1DM in offspring. The results from a similar study in a Finnish cohort conducted by Kilkkinen et al. found no association between maternal antibiotic use during pregnancy and development of T1DM in their children [178]. However, they did find a borderline risk associated with maternal use of few broad-spectrum antibiotics before pregnancy unlike in our study where we found no such association. The findings from studies that have evaluated the childhood antibiotic use and disease development has produced conflicting results [228–230]. Although there has been proof of dysbiosis induced T1DM available from animal models, the conflicting findings from human epidemiological studies instil concerns about the translational validity of those findings.

In contrast to our findings from the previous section on antenatal antibiotics and celiac disease, the use of any specific class of antibiotic did not alter the outcome. Although studies have identified underlying dysbiosis among T1DM children compared to T1DM naïve children, the results are inconclusive of whether the dysbiosis is a cause or consequence [217]. Our findings on the protective influence of birth in a lower income family, increasing parity and presence of other children in the house are indicators that childhood dysbiosis might have a causal influence. Interestingly, these covariates, unlike mode of delivery or antibiotic exposure, are influences that persist in the surrounding of a growing child for a longer duration. Our overall findings on childhood T1DM are suggestive of a lack of an association between maternal antibiotic exposure and disease development in their offspring.

4.4. Type II Diabetes Mellitus

Unlike type 1 diabetes where an immune component is involved, type 2 diabetes is associated with insulin resistance or hyposecretion of insulin by pancreatic β-cells or excess glucagon production. In comparison to other childhood diseases we studied, the magnitude of maternal hyperglycemia had a powerful impact on the development of childhood T2DM. Numerous studies have indicated that intrauterine exposure to maternal hyperglycemia predisposes their offspring to develop obesity and insulin resistance in their childhood [231, 232]. There was an evident gender polymorphism in our population, with male gender having a protective influence on the development of T2DM. This finding complies with the data from the existing literature which indicates a greater risk for pediatric T2DM among females compared to males [233]. In contravention to the belief that urban residence and higher income quintiles are risk factors for the development of chronic diseases, our findings are indicative of the opposite.

Interestingly, most children who develop T2DM in the province belong to the lowest income quintile and rural residence. Although the available data did not contain information on the race of the mother-infant dyads, it is possible that these findings are a reflection of the very high disease prevalence among First Nation (FN) children [234]. Premature birth, especially before 35 weeks gestational age and low birth weight infants are considered to precipitate insulin resistance, and the risk of T2DM has been found to be much higher among such children. Even though our findings on premature delivery before 35 weeks gestational age comply with the available evidence, the protective influence associated with lower birth weight is quite the opposite of what is expected. This might be because both high birth weight (HBW) and (although controversial) low birth weight

(LBW), independent of gestational age are risk factors for the development of childhood obesity and subsequently T2DM [235]. We used birth weight >4500 grams as the reference group and a meta-analysis by Yu et al. indicated that HBW rather than LBW was a bigger risk factor for childhood obesity [236]. C-section delivery, which is known to alter the infant gut microbiota composition was associated with a modestly elevated T2DM event rate. Although higher maternal age is a known risk factor for the development of gestational diabetes, which also increases the risk for T2DM in the child, our findings were indicative of a protective influence due to unclear reasons. We found a three-fold increase in the event rate for T2DM among children who were not exclusively breastfed. Other long-term influences that play a role in shaping the gut microbiota of a developing child are the presence of other children in the household, which promotes microbial diversity in the domestic environment.

In contrast to this hypothesis, there was a borderline elevation of T2DM risk among children living in house having other children. It is hypothesized that the women of higher parity have greater risk of diabetes than those of lower parity due to higher caloric intake and lower activity [237]. Although this is not generalizable, this could be the reason that we see a borderline significance or absence of an expected protective effect associated with higher parity. Although our methodology did not consider the racial factors, the association between pediatric T2DM and higher birth order might be due to the higher prevalence of the disease among FN families, who have a higher birth rate than non-FN population [238]. Studies which compared the season of birth and development of type 2 diabetes produced inconsistent results, probably due to the variation in seasons and associated seasonal infections varying from region to region [239–241]. Although not comparable due to the

geographic seasonal variations, our finding of a lack of seasonal influence complies with the findings of Jensen et al. [242].

Though maternal antenatal antibiotic use had borderline significance before adjustment, the significance was lost after adjustment. Similar borderline, yet non-significant association was present when antibiotics were prescribed for children during the first three to six months of life. However, the risk associated with childhood antibiotic use was not adjusted and hence the findings are uninterpretable. There was lack of association between maternal pre-pregnancy or post-pregnancy antibiotic use before and after exposure. Although the overall antenatal antibiotic use did not significantly alter the outcome, there was a two-fold increase in the risk of T2DM in the offspring when three or more courses of antibiotics were prescribed during pregnancy. The increase in T2DM event rate among offspring of mothers who were exposed during the third trimester is indicative of a temporal gradient. Although these findings indicate that maternal antibiotic use during pregnancy is associated with increased risk of T2DM in infants, the underlying reason may not involve the transfer of a perturbed gut microbiota to the infant. Studies have identified that the use of fluoroquinolone causes dysglycemia due to insulin resistance associated with magnesium chelation. Although fluoroquinolone is a pregnancy class C drug and is not routinely used during pregnancy, it is possible that the consumption of fluoroquinolone could precipitate insulin resistance [243]. The deficiency of magnesium during pregnancy could be aggravated due to the administration of fluoroquinolone and result in a hyperglycemic condition [244]. In our study, we found an elevated risk for T2DM among infants whose mothers were exposed to the group of antibiotics comprising of tetracycline, fluoroquinolone and other antibacterials. There is also a possibility of reverse causation

with diabetic mothers being more prone to urinary tract infections and thereby leading to a higher the use of nitrofurantoin and fluoroquinolone (both present in the same group). However, the first line therapies for urinary tract infection during pregnancy is beta-lactam antibiotics, and a lack of association with either penicillin or beta-lactam antibiotic use during pregnancy contradicts the possibility of reverse causation. It is possible that higher infection load during pregnancy affects the glycemic balance due to the infection itself or as a result of antibiotic consumption. The underlying factor which influences the T2DM development in the child could either be a result of intra-uterine exposure to the higher glucose level in maternal blood or an outcome of the perturbed maternal microbiota being transferred to the infant. Although we are unable to pin-point the underlying mechanism that is involved, our overall findings are indicative of a greater risk for T2DM among children of mothers who are prescribed either multiple antibiotic courses, third-trimester use of antibiotics or some specific classes of antibiotics (ATC codes J01A, J01M or J01X).

4.5. Cholelithiasis

The intra-uterine environment is considered to be a major determinant in the development of childhood chronic diseases. In our study, we found that the presence of maternal cholelithiasis causes a two-fold increase in the event rate of cholelithiasis in their offspring. This indicates the strong role of genetic components such as defects in the ABCB4 genes in the development of this disease. Maternal genetic variation in genes coding for biliary transporters could be passed on to the infant, predisposing them to develop impaired biliary clearance and resultant gallstone formation during their childhood [245–247]. Among adults, there is an evident sexual dimorphism with females having a higher risk than males due to hormonal variations and risk associated with pregnancy.

However, most studies have found a higher incidence among girls which might be a result of the pubertal changes in the second decade of life [248]. Although it is considered that the risk is equal among male and female children before puberty, our findings were indicative of a lesser risk among boys in comparison to girls. Children who lived in urban areas were found to be at lower risk than children from rural locations. Also, children who were born into less affluent families had a greater risk. The North American Aboriginal population is considered to be at a disproportionately higher risk for several liver conditions that causes cholelithiasis. Although genetic and dietary factors are important in the development of conditions like adult idiopathic cholelithiasis, liver diseases as a result of alcohol abuse also play a very significant role [249–251]. We hypothesize that the increased risk associated with rural residence and lower income quintile might be a reflection of the higher disease prevalence among the FN population in the province. The FN population is considered to conceive at a much younger age than the other communities, and this might again have influenced the moderately higher risk of cholelithiasis among offspring of mothers who were less than 20 years at parturition [252]. In comparison to exclusively breastfed infants, those who were formula fed had a much greater risk, which could be attributed to the protective effect associated with breastfeeding. Although the number of children in a family is protective from the perspective of old friend hypothesis and hygiene hypothesis, the slight elevation in the risk in our population might be due to reasons similar to those discussed in the section for T2DM.

Childhood antibiotic use in the initial 6 months of life, which is a crucial period for the development and maturation of both gut microbiota and immune system were only having a borderline influence on the outcome. Maternal antibiotic use before pregnancy,

during pregnancy and post-pregnancy, was associated with an elevated risk of cholelithiasis in their offspring. Although this does not eliminate the risk associate with maternal antibiotic use during pregnancy, the risk is not exclusive. The increase in risk irrespective of the timing of antibiotic is highly indicative of a confounding influence associated with maternal susceptibility to infection. Due to the non-exclusivity of the risk, it is possible that the dose-response relationship that is evident in our findings will be a consequence of the higher maternal propensity for infections.

Nevertheless, antibiotic use irrespective of the maternal infection susceptibility is a strong risk factor for dysbiosis and when they are used cumulatively by susceptible mothers, might persist in their body and get transferred to the infant's gut through breastmilk. However, if antibiotic-induced perturbation during the third trimester is involved in the disease development, exposure during third trimester should have a much higher event rate than the other trimesters. The overall results therefore indicate that maternal infection propensity drives maternal antibiotic use and childhood cholelithiasis development. Nevertheless, our findings cannot exclusively determine if infection susceptibility in the absence of antibiotic use might result in the development of cholelithiasis and hence caution needs to be exercised while prescribing antibiotics to pregnant women.

4.6. Strengths and Limitations

Several common issues in longitudinal population database analyses limit this study. The time of acquiring a disease and being diagnosed with a disorder is not necessarily the same. This study could not determine when chronic diseases had developed since outcome measures were based only on diagnoses.

We could not control for all confounding factors in our analyses as information on maternal dietary habits, breastfeeding after discharge from hospital and other environmental influences which could modify the disease development [253]. These factors may be responsible for some discrepancies in our results. We acknowledge that we did not assess some important chronic diseases such as inflammatory bowel disease, connective tissue disorders, cystic fibrosis, sickle cell anemia, epilepsy or cancer in this study. Furthermore, untreated or undiagnosed chronic disorders could not be captured in our analysis.

Reliability of data in medical, hospital, and nursing home files is a challenge as variation in training and supervision can cause differences in coding styles, affect the accuracy and completeness of diagnosis data and eventually produce unreliable statistics [254, 255]. Medical claims database, the diagnosis code is recorded at the three-digit level and it is possible that we overestimated the outcomes such as celiac disease and cholelithiasis. The lack of operational definition for each disease also compromises the quality of data generated by the ICD coding system [254]. Incorrect diagnoses made by a physician is also possible. Additionally, coding errors are possible when entering the codes in the databases, although data entry staff are well trained to decrease the chance of this type of error. It is also possible that visits to salaried primary care physicians may not all

be captured in the Repository. Most of Manitoba's physicians are fee-for-service paid, but others are salaried. Katz et al. reported that up to one-third of all visits to salaried primary care physicians are not captured in the Repository [256]. Their further work found a few difference between these two type of physicians billing reports [257]. However, this issue was more dominant in rural salaried physicians who were more susceptible to missing billings than Winnipeg-based physicians. This is a real concern in the Northern Health Region where most primary care physicians are salaried. In addition, the DPIN data does not cover the prescriptions data from nursing stations. Therefore, our findings cannot be generalized to all of Manitoba's children.

The database lacked the information regarding the indication for which the antibiotic was prescribed. Misclassification is also possible as 98% of deaths and out-of-province moves (migration) are recorded within one year after the event. This delay may not reflect current practices [254]. The time lag is also evident for 12% of Manitobans when moving among postal codes annually and almost half of these codes change after late reporting [258]. It is possible that misclassification occurs at the exposure level since the related databases did not capture antibiotics administered in the hospital. Therefore, confirming the consumption of filled prescriptions is impossible. Main outcome misclassification is also possible as some outcome measures may not be valid for younger children.

Additionally, covariate misclassification is possible. Using administrative data for breastfeeding initiation may misclassify dyads' infant feeding status. If a mother attempts to breastfeed but fails due to latch during the hospital stay; such a dyad is classified as "breastfed" in administrative data [259]. Most of the outcome measures were valid for younger children. We were looking at a large time range but this limited the children born

later in this cohort. We could not check the impact of breastfeeding on infant microbiota and transmission of antibiotics in breastmilk because a long follow-up (for about one year post-birth) is required. When the mother leaves the hospital, the duration of breastfeeding is unknown. Exclusive breastfeeding as a variable is also not available in the administrative data. There is information on breastfeeding initiation in the hospital (exclusive breastfeeding, formula-fed, mixed) but once the baby is discharged, it is not clear if they are exclusively breastfed or not.

Prescription data only starts in 1995 (April 1, 1995). This means that births early in our study period would not have completed exposure information before 1996. The result of the study cannot be generalized to individuals who are insured federally (military and federal inmates) or not eligible for coverage in Manitoba Health Insurance Registry. Moreover, any relevant contacts for those who have left the province or moved into the province sometime after birth while being out of the province were permanently missing [260].

We measured income at the area-level and evidence supports that area-level measurement can be a good indicator of individual-level socio-economic status [259, 261].

Despite the limitations listed above, this study will offer a wealth of information and a population-based perspective of Manitoba's children's chronic diseases with a potential risk factor. Our study using the MCHP database effectively captured the majority of the children who were born in Manitoba between 1996 and 2012 and helped us independently record the necessary healthcare information of the children. The advantage of such registry-based study is the inclusiveness, flexibility as well as flexibility, and also helps eliminate recall bias (that can affect the calculation of temporal gradient and dose-response

relationship) associated with prospective self-report or questionnaire-based studies. Additionally, we were able to adjust for both fetal and infant antibiotic use. This is an important adjustment as there is a link between maternal and infant healthcare utilization while each could be independently correlated to childhood chronic diseases [262]. Our study is also unique to assess seven chronic disease associations with antenatal exposure. Finally, our study provides new evidence to identify some new risk factors for childhood chronic diseases including maternal chronic disease, urban residence, preterm birth, and lower birth order.

Chapter 5. CONCLUSION

Our study was the first of its kind that evaluated the possible association of antenatal antibiotics with the development of multiple chronic diseases simultaneously in a population cohort. We found that the antenatal use of antibiotic was associated with an increased risk of development of severe allergy, celiac disease and cholelithiasis. Although an evident dose-response relationship between antibiotic exposure and the development of these diseases indicated a causative role, the sensitivity analysis showed that the risk was non-exclusive to exposure during pregnancy. The association of maternal antibiotic use 9-months before and 9-months after parturition indicates the possibility that the maternal susceptibility to infections might be acting as a confounder. Also, children who had early life antibiotic exposure (especially in the first 6 months) had an increased event rate compared to unexposed children. Although these are indications of an increased maternal physician-seeking behaviour, which positively correlates with both maternal and infant antibiotic use, the lack of an association between maternal physician visit frequency and the development of the diseases eliminate the possibility. Our findings, therefore, indicate that either the maternal susceptibility to infection or the resultant antibiotic use (irrespective of the timing concerning parturition) might be associated with the development of severe allergy, celiac disease and cholelithiasis in our Manitoban population cohort. The impact of antibiotic as a result of the perturbation in the maternal microbiota is transmitted to the infant either in-utero or during delivery and breast-feeding. The increased maternal antibiotic use might be having an utero-impact on the fetal microbiota, which subsequently interferes with the development of their immune system. Alternatively, the persistence of antibiotics in the maternal body because of binding to

maternal tissues could result in a vertical transmission during parturition or horizontal transmission through breastfeeding. However, if antibiotic exposure and not the maternal infection propensity were the cause, then the exposure during the third trimester, which is a crucial period for the microbiota transfer, would have had a stronger association. The absence of a temporal gradient (the lack of greater event rate among children whose mothers were exposed during the third trimester) indicates that maternal antibiotic exposure, as well as the disease outcome in the child, is an outcome of a higher propensity for infection in mother. The dose-response relationship might also be an outcome of higher risk of infection, which subsequently increases the antibiotic exposure during pregnancy. However, our study design was inadequate to test if maternal infection propensity, in the absence of resultant antibiotic exposure can increase the event rate in children. In the context of maternal antibiotics and childhood type 2 diabetes, the presence of a temporal gradient along with dose-response relationship is highly indicative of a causative influence. Though the role of a perturbed microbiota being transferred to childhood can only be hypothesized and not be proven using our study design, our findings indicate that multiple courses of antibiotics during pregnancy and exposure to antibiotics during the third trimester in mothers are associated with increased event rate in their offspring.

Our findings although indicative of the role of maternal infection propensity in the development of childhood severe allergy, celiac disease and cholelithiasis, cannot completely rule out the risk associated with antibiotic use. Moreover, type 2 diabetes risk was found to be higher among children of antibiotic exposed mothers. In the light of our finding, we emphasize that health care professionals be judicious and cautious when administering antibiotics to pregnant women.

REFERENCES

[1] Burns KH, Casey PH, Lyle RE, et al. Increasing Prevalence of Medically Complex Children in US Hospitals. *Pediatrics* 2010; 126: 638–646.

[2] Van Cleave J, Gortmaker SL, Perrin JM. Dynamics of obesity and chronic health conditions among children and youth. *JAMA* 2010; 303: 623–630.

[3] Gortmaker SL, Sappenfield W. Chronic childhood disorders: prevalence and impact. *Pediatr Clin North Am* 1984; 31: 3–18.

[4] van Dyck PC, Kogan MD, McPherson MG, et al. Prevalence and characteristics of children with special health care needs. *Arch Pediatr Adolesc Med* 2004; 158: 884–890.

[5] Cleave JV, Gortmaker SL, Perrin JM. Dynamics of Obesity and Chronic Health Conditions Among Children and Youth. *JAMA* 2010; 303: 623–630.

[6] WHO | Preventing disease through healthy environments: a global assessment of the burden of disease from environmental risks. *WHO*, http://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/ (accessed 2 March 2018).

[7] Martinez YJ, Ercikan K. Chronic illnesses in Canadian children: what is the effect of illness on academic achievement, and anxiety and emotional disorders? *Child Care Health Dev* 2009; 35: 391–401.

[8] Chronic Disease Indicator Framework, Fall 2014 Edition, http://www.phac-aspc.gc.ca/publicat/hpcdp-pspmc/34-4/assets/pdf/CDIC_MCC_Vol34_4_12_CDIIF_eng.pdf (2014, accessed 3 February 2018).

[9] Asher MI, Montefort S, Björkstén B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet Lond Engl* 2006; 368: 733–743.

[10] Woolcock AJ, Peat JK. Evidence for the increase in asthma worldwide. *Ciba Found Symp* 1997; 206: 122–134; discussion 134-139, 157–159.

[11] Soto-Quiros ME, Soto-Martinez M, Hanson LA. Epidemiological studies of the very high prevalence of asthma and related symptoms among school children in

Costa Rica from 1989 to 1998. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol* 2002; 13: 342–349.

[12] Williams HC. Is the prevalence of atopic dermatitis increasing? *Clin Exp Dermatol* 1992; 17: 385–391.

[13] Farrokhyar F, Swarbrick ET, Irvine EJ. A critical review of epidemiological studies in inflammatory bowel disease. *Scand J Gastroenterol* 2001; 36: 2–15.

[14] Poser S, Stickel B, Krtsch U, et al. Increasing incidence of multiple sclerosis in South Lower Saxony, Germany. *Neuroepidemiology* 1989; 8: 207–213.

[15] Eder W, Ege MJ, von Mutius E. The Asthma Epidemic. *N Engl J Med* 2006; 355: 2226–2235.

[16] Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299: 1259–1260.

[17] Leibowitz U, Antonovsky A, Medalie JM, et al. Epidemiological study of multiple sclerosis in Israel. II. Multiple sclerosis and level of sanitation. *J Neurol Neurosurg Psychiatry* 1966; 29: 60–68.

[18] Gerrard JW, Geddes CA, Reggin PL, et al. Serum IgE levels in white and metis communities in Saskatchewan. *Ann Allergy* 1976; 37: 91–100.

[19] Barker DJP, Osmond C, Golding J, et al. Acute appendicitis and bathrooms in three samples of British children. *Br Med J Clin Res Ed* 1988; 296: 956–958.

[20] Barker DJP, Morris J. Acute appendicitis, bathrooms, and diet in Britain and Ireland. *Br Med J Clin Res Ed* 1988; 296: 953–955.

[21] Holt PG. A potential vaccine strategy for asthma and allied atopic diseases during early childhood. *Lancet Lond Engl* 1994; 344: 456–458.

[22] Romagnani S. Human TH1 and TH2 Subsets: Regulation of Differentiation and Role in Protection and Immunopathology. *Int Arch Allergy Immunol* 1992; 98: 279–285.

[23] Allen JE, Maizels RM. Th1-Th2: reliable paradigm or dangerous dogma? *Immunol Today* 1997; 18: 387–392.

[24] Staines A, Bodansky HJ, McKinney PA, et al. Small area variation in the incidence of childhood insulin-dependent diabetes mellitus in Yorkshire, UK: links with overcrowding and population density. *Int J Epidemiol* 1997; 26: 1307–1313.

[25] Patterson CC, Carson DJ, Hadden DR. Epidemiology of childhood IDDM in Northern Ireland 1989-1994: low incidence in areas with highest population density and most household crowding. Northern Ireland Diabetes Study Group. *Diabetologia* 1996; 39: 1063–1069.

[26] von Mutius E, Martinez FD, Fritzsch C, et al. Prevalence of asthma and atopy in two areas of West and East Germany. *Am J Respir Crit Care Med* 1994; 149: 358–364.

[27] Bodansky HJ, Staines A, Stephenson C, et al. Evidence for an environmental effect in the aetiology of insulin dependent diabetes in a transmigratory population. *BMJ* 1992; 304: 1020–1022.

[28] Blanchard JF, Bernstein CN, Wajda A, et al. Small-area Variations and Sociodemographic Correlates for the Incidence of Crohn's Disease and Ulcerative Colitis. *Am J Epidemiol* 2001; 154: 328–335.

[29] Addo-Yobo EOD, Woodcock A, Allotey A, et al. Exercise-Induced Bronchospasm and Atopy in Ghana: Two Surveys Ten Years Apart. *PLoS Med*; 4. Epub ahead of print February 2007. DOI: 10.1371/journal.pmed.0040070.

[30] Braun-Fahrländer C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347: 869–877.

[31] Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002; 288: 963–972.

[32] Veckman V, Miettinen M, Pirhonen J, et al. Streptococcus pyogenes and Lactobacillus rhamnosus differentially induce maturation and production of Th1-type cytokines and chemokines in human monocyte-derived dendritic cells. *J Leukoc Biol* 2004; 75: 764–771.

[33] Prescott SL, Macaubas C, Smallacombe T, et al. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet Lond Engl* 1999; 353: 196–200.

[34] Prescott SL, Macaubas C, Smallacombe T, et al. Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 1998; 28 Suppl 5: 39–44; discussion 50-51.

[35] Bach J-F. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; 347: 911–920.

[36] Stene LC, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. *Lancet Lond Engl* 2001; 357: 607–608.

[37] Yazdanbakhsh M, Kremsner PG, van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; 296: 490–494.

[38] Weiner HL. Induction and mechanism of action of transforming growth factor-beta-secreting Th3 regulatory cells. *Immunol Rev* 2001; 182: 207–214.

[39] McHugh RS, Whitters MJ, Piccirillo CA, et al. CD4(+)CD25(+) immunoregulatory T cells: gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. *Immunity* 2002; 16: 311–323.

[40] Roncarolo MG, Bacchetta R, Bordignon C, et al. Type 1 T regulatory cells. *Immunol Rev* 2001; 182: 68–79.

[41] Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol* 2003; 4: 330–336.

[42] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003; 299: 1057–1061.

[43] Scholz C, Patton KT, Anderson DE, et al. Expansion of autoreactive T cells in multiple sclerosis is independent of exogenous B7 costimulation. *J Immunol Baltim Md 1950* 1998; 160: 1532–1538.

[44] Danke NA, Koelle DM, Yee C, et al. Autoreactive T cells in healthy individuals. *J Immunol Baltim Md 1950* 2004; 172: 5967–5972.

[45] Baecher-Allan C, Hafler DA. Suppressor T Cells in Human Diseases. *J Exp Med* 2004; 200: 273–276.

[46] Itoh M, Takahashi T, Sakaguchi N, et al. Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. *J Immunol Baltim Md 1950* 1999; 162: 5317–5326.

[47] Belkaid Y, Piccirillo CA, Mendez S, et al. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. *Nature* 2002; 420: 502–507.

[48] Bloomfield S, Stanwell-Smith R, Crevel R, et al. Too clean, or not too clean: the Hygiene Hypothesis and home hygiene. *Clin Exp Allergy* 2006; 36: 402–425.

[49] Benn CS, Melbye M, Wohlfahrt J, et al. Cohort study of sibling effect, infectious diseases, and risk of atopic dermatitis during first 18 months of life. *BMJ* 2004; 328: 1223.

[50] Bremner SA, Carey IM, DeWilde S, et al. Infections presenting for clinical care in early life and later risk of hay fever in two UK birth cohorts. *Allergy* 2008; 63: 274–283.

[51] Kohashi O, Kohashi Y, Takahashi T, et al. Reverse effect of gram-positive bacteria vs. gram-negative bacteria on adjuvant-induced arthritis in germfree rats. *Microbiol Immunol* 1985; 29: 487–497.

[52] Moudgil KD, Kim E, Yun OJ, et al. Environmental modulation of autoimmune arthritis involves the spontaneous microbial induction of T cell responses to regulatory determinants within heat shock protein 65. *J Immunol Baltim Md 1950* 2001; 166: 4237–4243.

[53] Sudo N, Yu X-N, Aiba Y, et al. An oral introduction of intestinal bacteria prevents the development of a long-term Th2-skewed immunological memory induced by neonatal antibiotic treatment in mice. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2002; 32: 1112–1116.

[54] Verdù EF, Bercik P, Cukrowska B, et al. Oral administration of antigens from intestinal flora anaerobic bacteria reduces the severity of experimental acute colitis in BALB/c mice. *Clin Exp Immunol* 2000; 120: 46–50.

[55] Schultz M, Veltkamp C, Dieleman LA, et al. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm Bowel Dis* 2002; 8: 71–80.

[56] Babu S, Blauvelt CP, Kumaraswami V, et al. Regulatory networks induced by live parasites impair both Th1 and Th2 pathways in patent lymphatic filariasis: implications for parasite persistence. *J Immunol Baltim Md 1950* 2006; 176: 3248–3256.

[57] Hadley C. Should auld acquaintance be forgot... *EMBO Rep* 2004; 5: 1122–1124.

[58] Karimi K, Inman MD, Bienenstock J, et al. Lactobacillus reuteri-induced regulatory T cells protect against an allergic airway response in mice. *Am J Respir Crit Care Med* 2009; 179: 186–193.

[59] Rook GAW. Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology* 2009; 126: 3–11.

[60] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* 2009; 9: 313–323.

[61] Smits HH, Engering A, van der Kleij D, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. *J Allergy Clin Immunol* 2005; 115: 1260–1267.

[62] Hjern A, Rasmussen F, Hedlin G. Age at adoption, ethnicity and atopic disorder: a study of internationally adopted young men in Sweden. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol* 1999; 10: 101–106.

[63] Rook GAW, Lowry CA, Raison CL. Microbial 'Old Friends', immunoregulation and stress resilience. *Evol Med Public Health* 2013; 2013: 46–64.

[64] Baquero F, Nombela C. The microbiome as a human organ. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis* 2012; 18 Suppl 4: 2–4.

[65] Glendinning L, Free A. Supra-organismal interactions in the human intestine. *Front Cell Infect Microbiol*; 4. Epub ahead of print 23 April 2014. DOI: 10.3389/fcimb.2014.00047.

[66] Bäckhed F, Ley RE, Sonnenburg JL, et al. Host-bacterial mutualism in the human intestine. *Science* 2005; 307: 1915–1920.

[67] Gill SR, Pop M, DeBoy RT, et al. Metagenomic Analysis of the Human Distal Gut Microbiome. *Science* 2006; 312: 1355–1359.

[68] Bianconi E, Piovesan A, Facchin F, et al. An estimation of the number of cells in the human body. *Ann Hum Biol* 2013; 40: 463–471.

[69] Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* 2016; 14: e1002533.

[70] Liu Y, Zhang L, Wang X, et al. Similar Fecal Microbiota Signatures in Patients With Diarrhea-Predominant Irritable Bowel Syndrome and Patients With Depression. *Clin Gastroenterol Hepatol* 2016; 14: 1602-1611.e5.

[71] Tap J, Derrien M, Törnblom H, et al. Identification of an Intestinal Microbiota Signature Associated With Severity of Irritable Bowel Syndrome. *Gastroenterology* 2017; 152: 111-123.e8.

[72] Morgan XC, Huttenhower C. Chapter 12: Human Microbiome Analysis. *PLoS Comput Biol*; 8. Epub ahead of print 27 December 2012. DOI: 10.1371/journal.pcbi.1002808.

[73] NIH Human Microbiome Project - About the Human Microbiome, https://hmpdacc.org/hmp/overview/ (accessed 17 March 2018).

[74] Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature* 2007; 449: 804–810.

[75] Bengmark S. Ecological control of the gastrointestinal tract. The role of probiotic flora. *Gut* 1998; 42: 2–7.

[76] Guarner F, Malagelada J-R. Gut flora in health and disease. *The Lancet* 2003; 361: 512–519.

[77] Sears CL. A dynamic partnership: Celebrating our gut flora. *Anaerobe* 2005; 11: 247–251.

[78] Guinane CM, Cotter PD. Role of the gut microbiota in health and chronic gastrointestinal disease: understanding a hidden metabolic organ. *Ther Adv Gastroenterol* 2013; 6: 295–308.

[79] Possemiers S, Bolca S, Verstraete W, et al. The intestinal microbiome: a separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. *Fitoterapia* 2011; 82: 53–66.

[80] O'Hara AM, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; 7: 688–693.

[81] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012; 486: 207–214.

[82] Tissier H. *Recherches sur la flore intestinale des nourrissons:(état normal et pathologique)*. Doctoral Dissertation, University of Paris, 1900.

[83] Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999; 69: 1035S-1045S.

[84] Bearfield C, Davenport ES, Sivapathasundaram V, et al. Possible association between amniotic fluid micro-organism infection and microflora in the mouth. *BJOG Int J Obstet Gynaecol* 2002; 109: 527–533.

[85] Jiménez E, Fernández L, Marín ML, et al. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol* 2005; 51: 270–274.

[86] Rautava S, Collado MC, Salminen S, et al. Probiotics modulate host-microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology* 2012; 102: 178–184.

[87] Steel JH, O'Donoghue K, Kennea NL, et al. Maternal origin of inflammatory leukocytes in preterm fetal membranes, shown by fluorescence in situ hybridisation. *Placenta* 2005; 26: 672–677.

[88] Jiménez E, Marín ML, Martín R, et al. Is meconium from healthy newborns actually sterile? *Res Microbiol* 2008; 159: 187–193.

[89] Rescigno M, Urbano M, Valzasina B, et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001; 2: 361–367.

[90] Vazquez-Torres A, Jones-Carson J, Bäumler AJ, et al. Extraintestinal dissemination of Salmonella by CD18-expressing phagocytes. *Nature* 1999; 401: 804–808.

[91] Azad MB, Konya T, Maughan H, et al. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Can Med Assoc J* 2013; cmaj.121189.

[92] Biasucci G, Benenati B, Morelli L, et al. Cesarean delivery may affect the early biodiversity of intestinal bacteria. *J Nutr* 2008; 138: 1796S-1800S.

[93] Dominguez-Bello MG, Costello EK, Contreras M, 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: 11971–11975.

[94] Eggesbø M, Botten G, Stigum H, et al. Is delivery by cesarean section a risk factor for food allergy? *J Allergy Clin Immunol* 2003; 112: 420–426.

[95] Li Y, Caufield PW, Dasanayake AP, et al. Mode of delivery and other maternal factors influence the acquisition of Streptococcus mutans in infants. *J Dent Res* 2005; 84: 806–811.

[96] Aagaard K, Riehle K, Ma J, et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. *PloS One* 2012; 7: e36466.

[97] Cabrera-Rubio R, Collado MC, Laitinen K, et al. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr* 2012; 96: 544–551.

[98] Hunt KM, Foster JA, Forney LJ, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. *PloS One* 2011; 6: e21313.

[99] Million M, Tidjani Alou M, Khelaifia S, et al. Increased Gut Redox and Depletion of Anaerobic and Methanogenic Prokaryotes in Severe Acute Malnutrition. *Sci Rep* 2016; 6: 26051.

[100] Wall R, Ross R., Ryan C., et al. Role of Gut Microbiota in Early Infant Development. *Clin Med Pediatr* 2009; 3: 45–54.

[101] Lewis ZT, Mills DA. Differential Establishment of Bifidobacteria in the Breastfed Infant Gut. *Nestle Nutr Inst Workshop Ser* 2017; 88: 149–159.

[102] Monteagudo-Mera A, Arthur JC, Jobin C, et al. High purity galacto-oligosaccharides (GOS) enhance specific Bifidobacterium species and their metabolic activity in the mouse gut microbiome. *Benef Microbes* 2016; 7: 247–264.

[103] Tannock GW, Lee PS, Wong KH, et al. Why Don't All Infants Have Bifidobacteria in Their Stool? *Front Microbiol*; 7. Epub ahead of print 31 May 2016. DOI: 10.3389/fmicb.2016.00834.

[104] Makino H, Kushiro A, Ishikawa E, et al. Mother-to-Infant Transmission of Intestinal Bifidobacterial Strains Has an Impact on the Early Development of Vaginally Delivered Infant's Microbiota. *PLoS ONE*; 8. Epub ahead of print 14 November 2013. DOI: 10.1371/journal.pone.0078331.

[105] Makino H, Kushiro A, Ishikawa E, et al. Transmission of intestinal Bifidobacterium longum subsp. longum strains from mother to infant, determined by multilocus sequencing typing and amplified fragment length polymorphism. *Appl Environ Microbiol* 2011; 77: 6788–6793.

[106] Levy M, Kolodziejczyk AA, Thaiss CA, et al. Dysbiosis and the immune system. *Nat Rev Immunol* 2017; 17: 219–232.

[107] Chow J, Mazmanian SK. A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host Microbe* 2010; 7: 265–276.

[108] Stecher B, Maier L, Hardt W-D. 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. *Nat Rev Microbiol* 2013; 11: 277–284.

[109] Byndloss MX, Olsan EE, Rivera-Chávez F, et al. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science* 2017; 357: 570–575.

[110] Shin N-R, Whon TW, Bae J-W. Proteobacteria: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 2015; 33: 496–503.

[111] Bergman EN. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* 1990; 70: 567–590.

[112] Krajmalnik-Brown R, Ilhan Z-E, Kang D-W, et al. Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract Off Publ Am Soc Parenter Enter Nutr* 2012; 27: 201–214.

[113] De Vadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* 2014; 156: 84–96.

[114] Cani PD. Gut microbiota - at the intersection of everything? *Nat Rev Gastroenterol Hepatol* 2017; 14: 321–322.

[115] LeBlanc JG, Milani C, de Giori GS, et al. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr Opin Biotechnol* 2013; 24: 160–168.

[116] Kleerebezem M, Vaughan EE. Probiotic and Gut Lactobacilli and Bifidobacteria: Molecular Approaches to Study Diversity and Activity. *Annu Rev Microbiol* 2009; 63: 269–290.

[117] Arumugam M, Raes J, Pelletier E, et al. Enterotypes of the human gut microbiome. *Nature* 2011; 473: 174–180.

[118] Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; 334: 105–108.

[119] David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014; 505: 559–563.

[120] Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* 2014; 12: 661–672.

[121] Magee E. A nutritional component to inflammatory bowel disease: the contribution of meat to fecal sulfide excretion. *Nutr Burbank Los Angel Cty Calif* 1999; 15: 244–246.

[122] Mazmanian SK, Liu CH, Tzianabos AO, et al. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; 122: 107–118.

[123] Sekirov I, Tam NM, Jogova M, et al. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. *Infect Immun* 2008; 76: 4726–4736.

[124] Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. *Semin Immunol* 2007; 19: 59–69.

[125] Munyaka PM, Eissa N, Bernstein CN, et al. Antepartum Antibiotic Treatment Increases Offspring Susceptibility to Experimental Colitis: A Role of the Gut Microbiota. *PLoS ONE*; 10. Epub ahead of print 25 November 2015. DOI: 10.1371/journal.pone.0142536.

[126] Abrams GD, Bauer H, Sprinz H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Lab Investig J Tech Methods Pathol* 1963; 12: 355–364.

[127] Abrams GD, Bishop JE. Effect of the normal microbial flora on gastrointestinal motility. *Proc Soc Exp Biol Med Soc Exp Biol Med N Y N* 1967; 126: 301–304.

[128] Gustafsson BE, Midtvedt T, Strandberg K. Effects of microbial contamination on the cecum enlargement of germfree rats. *Scand J Gastroenterol* 1970; 5: 309–314.

[129] El Aidy S, Hooiveld G, Tremaroli V, et al. The gut microbiota and mucosal homeostasis: colonized at birth or at adulthood, does it matter? *Gut Microbes* 2013; 4: 118–124.

[130] Falk PG, Hooper LV, Midtvedt T, et al. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. *Microbiol Mol Biol Rev MMBR* 1998; 62: 1157–1170.

[131] Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; 139: 485–498.

[132] Chong CYL, Bloomfield FH, O'Sullivan JM. Factors Affecting Gastrointestinal Microbiome Development in Neonates. *Nutrients* 2018; 10: 274.

[133] Azad MB, Konya T, Persaud RR, 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 Int J Obstet Gynaecol* 2016; 123: 983–993.

[134] Rutayisire E, Huang K, Liu Y, et al. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. *BMC Gastroenterol*; 16. Epub ahead of print 30 July 2016. DOI: 10.1186/s12876-016-0498-0.

[135] Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat Med* 2016; 22: 250–253.

[136] Potential Restoration of the Infant Microbiome - Full Text View - ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT02407184 (accessed 24 March 2018).

[137] Penders J, Thijs C, Vink C, et al. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006; 118: 511–521.

[138] Jost T, Lacroix C, Braegger C, et al. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. *Br J Nutr* 2013; 110: 1253–1262.

[139] Walker WA, Iyengar RS. Breast milk, microbiota, and intestinal immune homeostasis. *Pediatr Res* 2014; 77: 220–228.

[140] Marcobal A, Barboza M, Sonnenburg ED, et al. Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. *Cell Host Microbe* 2011; 10: 507–514.

[141] Stark PL, Lee A. Clostridia isolated from the feces of infants during the first year of life. *J Pediatr* 1982; 100: 362–365.

[142] Veereman-Wauters G, Staelens S, Van de Broek H, et al. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. *J Pediatr Gastroenterol Nutr* 2011; 52: 763–771.

[143] Thompson AL, Monteagudo-Mera A, Cadenas MB, et al. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. *Front Cell Infect Microbiol* 2015; 5: 3.

[144] Chai G, Governale L, McMahon AW, et al. Trends of outpatient prescription drug utilization in US children, 2002-2010. *Pediatrics* 2012; 130: 23–31.

[145] Vangay P, Ward T, Gerber JS, et al. Antibiotics, Pediatric Dysbiosis, and Disease. *Cell Host Microbe* 2015; 17: 553–564.

[146] Hersh AL, Shapiro DJ, Pavia AT, et al. Antibiotic prescribing in ambulatory pediatrics in the United States. *Pediatrics* 2011; 128: 1053–1061.

[147] Stokholm J, Schjørring S, Pedersen L, et al. Prevalence and predictors of antibiotic administration during pregnancy and birth. *PloS One* 2013; 8: e82932.

[148] Korpela K, Salonen A, Virta LJ, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun* 2016; 7: 10410.

[149] Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* 2011; 60: 49–54.

[150] Kozyrskyj AL, Bahreinian S, Azad MB. Early life exposures: impact on asthma and allergic disease. *Curr Opin Allergy Clin Immunol* 2011; 11: 400–406.

[151] Romano-Keeler J, Weitkamp J-H. Maternal influences on fetal microbial colonization and immune development. *Pediatr Res* 2015; 77: 189–195.

[152] Miyoshi J, Bobe AM, Miyoshi S, et al. Peripartum exposure to antibiotics promotes persistent gut dysbiosis, immune imbalance, and inflammatory bowel disease in genetically prone offspring. *Cell Rep* 2017; 20: 491–504.

[153] Erwin G. Zoetendal WM de V Antoon DL Akkermans, Wilma M Akkermans-van Vliet, J Arjan GM de Visser. The Host Genotype Affects the Bacterial Community in the Human Gastronintestinal Tract. *Microb Ecol Health Dis* 2001; 13: 129–134.

[154] Stewart JA, Chadwick VS, Murray A. Investigations into the influence of host genetics on the predominant eubacteria in the faecal microflora of children. *J Med Microbiol* 2005; 54: 1239–1242.

[155] Turnbaugh PJ, Quince C, Faith JJ, et al. Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. *Proc Natl Acad Sci U S A* 2010; 107: 7503–7508.

[156] Murphy K, Shea CAO, Ryan CA, et al. The Gut Microbiota Composition in Dichorionic Triplet Sets Suggests a Role for Host Genetic Factors. *PLOS ONE* 2015; 10: e0122561.

[157] Kovalovszki L, Villányi Z, Pataki I, et al. Isolation of aerobic bacteria from the placenta. *Acta Paediatr Acad Sci Hung* 1982; 23: 357–360.

[158] Perez-Muñoz ME, Arrieta M-C, Ramer-Tait AE, et al. A critical assessment of the "sterile womb" and "in utero colonization" hypotheses: implications for research on the pioneer infant microbiome. *Microbiome* 2017; 5: 48.

[159] Koren O, Goodrich JK, Cullender TC, et al. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* 2012; 150: 470–480.

[160] Romero R, Hassan SS, Gajer P, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome* 2014; 2: 4.

[161] Korpela K, Blakstad EW, Moltu SJ, et al. Intestinal microbiota development and gestational age in preterm neonates. *Sci Rep* 2018; 8: 2453.

[162] Collado MC, Isolauri E, Laitinen K, et al. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. *Am J Clin Nutr* 2010; 92: 1023–1030.

[163] Cox LM, Blaser MJ. Pathways in microbe-induced obesity. *Cell Metab* 2013; 17: 883–894.

[164] Land MH, Rouster-Stevens K, Woods CR, et al. Lactobacillus sepsis associated with probiotic therapy. *Pediatrics* 2005; 115: 178–181.

[165] Ma J, Prince AL, Bader D, et al. High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nat Commun* 2014; 5: 3889.

[166] Bookstaver PB, Bland CM, Griffin B, et al. A Review of Antibiotic Use in Pregnancy. *Pharmacotherapy* 2015; 35: 1052–1062.

[167] Mitchell AA, Gilboa SM, Werler MM, et al. Medication use during pregnancy, with particular focus on prescription drugs: 1976-2008. *Am J Obstet Gynecol* 2011; 205: 51.e1–8.

[168] Gray LEK, O'Hely M, Ranganathan S, et al. The Maternal Diet, Gut Bacteria, and Bacterial Metabolites during Pregnancy Influence Offspring Asthma. *Front Immunol*; 8. Epub ahead of print 2017. DOI: 10.3389/fimmu.2017.00365.

[169] Murk W, Risnes KR, Bracken MB. Prenatal or Early-Life Exposure to Antibiotics and Risk of Childhood Asthma: A Systematic Review. *Pediatrics* 2011; 127: 1125–1138.

[170] Zhao D, Su H, Cheng J, et al. Prenatal antibiotic use and risk of childhood wheeze/asthma: A meta-analysis. *Pediatr Allergy Immunol Off Publ Eur Soc Pediatr Allergy Immunol* 2015; 26: 756–764.

[171] Timm S, Schlünssen V, Olsen J, et al. Prenatal antibiotics and atopic dermatitis among 18-month-old children in the Danish National Birth Cohort. *Clin Exp Allergy* 2017; 47: 929–936.

[172] Scher JU. The microbiome in celiac disease: Beyond diet-genetic interactions. *Cleve Clin J Med* 2016; 83: 228–230.

[173] Galipeau HJ, Rulli NE, Jury J, et al. Sensitization to gliadin induces moderate enteropathy and insulitis in nonobese diabetic-DQ8 mice. *J Immunol Baltim Md 1950* 2011; 187: 4338–4346.

[174] Mårild K, Ludvigsson J, Sanz Y, et al. Antibiotic exposure in pregnancy and risk of coeliac disease in offspring: a cohort study. *BMC Gastroenterol* 2014; 14: 75.

[175] Hu Y, Jin P, Peng J, et al. Different immunological responses to early-life antibiotic exposure affecting autoimmune diabetes development in NOD mice. *J Autoimmun* 2016; 72: 47–56.

[176] Hu Y, Peng J, Tai N, et al. Maternal Antibiotic Treatment Protects Offspring from Diabetes Development in Nonobese Diabetic Mice by Generation of Tolerogenic APCs. *J Immunol Baltim Md 1950* 2015; 195: 4176–4184.

[177] Goffau MC de, Luopajärvi K, Knip M, et al. Fecal Microbiota Composition Differs Between Children With β-Cell Autoimmunity and Those Without. *Diabetes* 2013; 62: 1238–1244.

[178] Kilkkinen A, Virtanen SM, Klaukka T, et al. Use of antimicrobials and risk of type 1 diabetes in a population-based mother–child cohort. *Diabetologia* 2006; 49: 66–70.

[179] Larsen N, Vogensen FK, Berg FWJ van den, et al. Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. *PLOS ONE* 2010; 5: e9085.

[180] Muñoz-Garach A, Diaz-Perdigones C, Tinahones FJ. Gut microbiota and type 2 diabetes mellitus. *Endocrinol Nutr Engl Ed* 2016; 63: 560–568.

[181] Trasande L, Blustein J, Liu M, et al. Infant antibiotic exposures and early-life body mass. *Int J Obes 2005* 2013; 37: 16–23.

[182] Chou CJ, Membrez M, Blancher F. Gut decontamination with norfloxacin and ampicillin enhances insulin sensitivity in mice. *Nestle Nutr Workshop Ser Paediatr Programme* 2008; 62: 127–137; discussion 137-140.

[183] Membrez M, Blancher F, Jaquet M, et al. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. *FASEB J Off Publ Fed Am Soc Exp Biol* 2008; 22: 2416–2426.

[184] Vrieze A, Van Nood E, Holleman F, et al. Transfer of Intestinal Microbiota From Lean Donors Increases Insulin Sensitivity in Individuals With Metabolic Syndrome. *Gastroenterology* 2012; 143: 913-916.e7.

[185] Reshetnyak VI. Concept of the pathogenesis and treatment of cholelithiasis. *World J Hepatol* 2012; 4: 18–34.

[186] Katsika D, Grjibovski A, Einarsson C, et al. Genetic and environmental influences on symptomatic gallstone disease: A Swedish study of 43,141 twin pairs. *Hepatology*; 41: 1138–1143.

[187] Tabibian JH, Varghese C, LaRusso NF, et al. The enteric microbiome in hepatobiliary health and disease. *Liver Int Off J Int Assoc Study Liver* 2016; 36: 480–487.

[188] Rudolph G, Gotthardt D, Klöters-Plachky P, et al. Influence of dominant bile duct stenoses and biliary infections on outcome in primary sclerosing cholangitis. *J Hepatol* 2009; 51: 149–155.

[189] Walker SK, Maki AC, Cannon RM, et al. Etiology and incidence of pediatric gallbladder disease. *Surgery* 2013; 154: 927–931; discussion 931-933.

[190] Stokholm J, Sevelsted A, Bønnelykke K, et al. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med* 2014; 2: 631–637.

[191] Blaser MJ, Bello MGD. Maternal antibiotic use and risk of asthma in offspring. *Lancet Respir Med* 2014; 2: e16.

[192] Loewen K, Monchka B, Mahmud SM, et al. Prenatal antibiotic exposure and childhood asthma: a population-based study. *Eur Respir J* 2018; 1702070.

[193] Concept: Income Quintiles - Child Health Income Quintiles, http://mchp-appserv.cpe.umanitoba.ca/viewConcept.php?printer=Y&conceptID=1161 (accessed 16 July 2018).

[194] Broe A, Pottegård A, Lamont RF, et al. Increasing use of antibiotics in pregnancy during the period 2000-2010: prevalence, timing, category, and demographics. *BJOG Int J Obstet Gynaecol* 2014; 121: 988–996.

[195] Petersen I, Gilbert R, Evans S, et al. Oral antibiotic prescribing during pregnancy in primary care: UK population-based study. *J Antimicrob Chemother* 2010; 65: 2238–2246.

[196] Andrade SE, Gurwitz JH, Davis RL, et al. Prescription drug use in pregnancy. *Am J Obstet Gynecol* 2004; 191: 398–407.

[197] Aagaard K, Ma J, Antony KM, et al. The placenta harbors a unique microbiome. *Sci Transl Med* 2014; 6: 237ra65.

[198] Jedrychowski W, Gałaś A, Whyatt R, et al. The prenatal use of antibiotics and the development of allergic disease in one year old infants. A preliminary study. *Int J Occup Med Environ Health* 2006; 19: 70–76.

[199] Mitre E, Susi A, Kropp LE, et al. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood. *JAMA Pediatr* 2018; e180315.

[200] McKeever TM, Lewis SA, Smith C, et al. The Importance of Prenatal Exposures on the Development of Allergic Disease. *Am J Respir Crit Care Med* 2002; 166: 827–832.

[201] Metsälä J, Lundqvist A, Virta LJ, et al. Mother's and Offspring's Use of Antibiotics and Infant Allergy to Cow's Milk. *Epidemiology* 2013; 24: 303.

[202] Jernberg C, Löfmark S, Edlund C, et al. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J* 2007; 1: 56–66.

[203] Dethlefsen L, Huse S, Sogin ML, et al. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* 2008; 6: e280.

[204] De La Cochetière MF, Durand T, Lepage P, et al. Resilience of the dominant human fecal microbiota upon short-course antibiotic challenge. *J Clin Microbiol* 2005; 43: 5588–5592.

[205] Mårild K, Ye W, Lebwohl B, et al. Antibiotic exposure and the development of coeliac disease: a nationwide case–control study. *BMC Gastroenterol* 2013; 13: 109.

[206] Salvatore S, Finazzi S, Radaelli G, et al. Prevalence of undiagnosed celiac disease in the parents of preterm and/or small for gestational age infants. *Am J Gastroenterol* 2007; 102: 168–173.

[207] Khashan AS, Henriksen TB, Mortensen PB, et al. The impact of maternal celiac disease on birthweight and preterm birth: a Danish population-based cohort study. *Hum Reprod* 2010; 25: 528–534.

[208] Namatovu F, Olsson C, Lindkvist M, et al. Maternal and perinatal conditions and the risk of developing celiac disease during childhood. *BMC Pediatr* 2016; 16: 77.

[209] Namatovu F, Lindkvist M, Olsson C, et al. Season and region of birth as risk factors for coeliac disease a key to the aetiology? *Arch Dis Child* 2016; 101: 1114–1118.

[210] Ivarsson A, Persson L, Nyström L, et al. Epidemic of coeliac disease in Swedish children. *Acta Paediatr*; 89: 165–171.

[211] Kokkonen J, Similä S, Vuolukka P. The incidence of coeliac disease and pyloric stenosis in children in Northern Finland. *Ann Clin Res* 1982; 14: 123–128.

[212] Thavagnanam S, Fleming J, Bromley A, et al. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy J Br Soc Allergy Clin Immunol* 2008; 38: 629–633.

[213] Hyde MJ, Mostyn A, Modi N, et al. The health implications of birth by Caesarean section. *Biol Rev Camb Philos Soc* 2012; 87: 229–243.

[214] Cardwell CR, Stene LC, Joner G, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia* 2008; 51: 726–735.

[215] Sevelsted A, Stokholm J, Bønnelykke K, et al. Cesarean section and chronic immune disorders. *Pediatrics* 2015; 135: e92-98.

[216] Mårild K, Kahrs CR, Tapia G, et al. Maternal Infections, Antibiotics, and Paracetamol in Pregnancy and Offspring Celiac Disease: A Cohort Study. *J Pediatr Gastroenterol Nutr* 2017; 64: 730–736.

[217] Knip M, Siljander H. The role of the intestinal microbiota in type 1 diabetes mellitus. *Nat Rev Endocrinol* 2016; 12: 154–167.

[218] Harjutsalo V, Reunanen A, Tuomilehto J. Differential Transmission of Type 1 Diabetes from Diabetic Fathers and Mothers to Their Offspring. *Diabetes* 2006; 55: 1517–1524.

[219] Hussen HI, Persson M, Moradi T. Maternal overweight and obesity are associated with increased risk of type 1 diabetes in offspring of parents without diabetes regardless of ethnicity. *Diabetologia* 2015; 58: 1464–1473.

[220] Lindell N, Carlsson A, Josefsson A, et al. Maternal obesity as a risk factor for early childhood type 1 diabetes: a nationwide, prospective, population-based case–control study. *Diabetologia* 2018; 61: 130–137.

[221] Gale EA, Gillespie KM. Diabetes and gender. *Diabetologia* 2001; 44: 3–15.

[222] Patterson CC, Waugh NR. Urban/rural and deprivational differences in incidence and clustering of childhood diabetes in Scotland. *Int J Epidemiol* 1992; 21: 108–117.

[223] Prel J-B du, Icks A, Grabert M, et al. Socioeconomic conditions and type 1 diabetes in childhood in North Rhine–Westphalia, Germany. *Diabetologia* 2007; 50: 720–728.

[224] Mayer EJ, Hamman RF, Gay EC, et al. Reduced risk of IDDM among breastfed children. The Colorado IDDM Registry. *Diabetes* 1988; 37: 1625–1632.

[225] Bruno G, Pagano G, Faggiano F, et al. Effect of Sardinian heritage on risk and age at onset of type 1 diabetes: a demographic case-control study of Sardinian migrants. *Int J Epidemiol* 2000; 29: 532–535.

[226] Vehling L, Chan D, McGavock J, et al. Exclusive breastfeeding in hospital predicts longer breastfeeding duration in Canada: Implications for health equity. *Birth Berkeley Calif*. Epub ahead of print 2 March 2018. DOI: 10.1111/birt.12345.

[227] Cardwell CR, Stene LC, Joner G, et al. Maternal Age at Birth and Childhood Type 1 Diabetes: A Pooled Analysis of 30 Observational Studies. *Diabetes* 2010; 59: 486–494.

[228] Clausen TD, Bergholt T, Bouaziz O, et al. Broad-Spectrum Antibiotic Treatment and Subsequent Childhood Type 1 Diabetes: A Nationwide Danish Cohort Study. *PLoS ONE*; 11. Epub ahead of print 25 August 2016. DOI: 10.1371/journal.pone.0161654.

[229] Hviid A, Svanström H. Antibiotic use and type 1 diabetes in childhood. *Am J Epidemiol* 2009; 169: 1079–1084.

[230] Fazeli Farsani S, Souverein PC, van der Vorst MMJ, et al. Population-based cohort study of anti-infective medication use before and after the onset of type 1 diabetes in children and adolescents. *Antimicrob Agents Chemother* 2014; 58: 4666–4674.

[231] Dabelea D. The predisposition to obesity and diabetes in offspring of diabetic mothers. *Diabetes Care* 2007; 30 Suppl 2: S169-174.

[232] Ge Z-J, Zhang C-L, Schatten H, et al. Maternal Diabetes Mellitus and the Origin of Non-Communicable Diseases in Offspring: The Role of Epigenetics. *Biol Reprod*; 90. Epub ahead of print 1 June 2014. DOI: 10.1095/biolreprod.114.118141.

[233] Zdravkovic V, Daneman D, Hamilton J. Presentation and course of Type 2 diabetes in youth in a large multi-ethnic city. *Diabet Med J Br Diabet Assoc* 2004; 21: 1144–1148.

[234] Amed S, Dean HJ, Panagiotopoulos C, et al. Type 2 Diabetes, Medication-Induced Diabetes, and Monogenic Diabetes in Canadian Children. *Diabetes Care* 2010; 33: 786–791.

[235] Power C, Li L, Manor O, et al. Combination of low birth weight and high adult body mass index: at what age is it established and what are its determinants? *J Epidemiol Community Health* 2003; 57: 969–973.

[236] Yu ZB, Han SP, Zhu GZ, et al. Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. *Obes Rev Off J Int Assoc Study Obes* 2011; 12: 525–542.

[237] Nicholson WK, Asao K, Brancati F, et al. Parity and Risk of Type 2 Diabetes: The Atherosclerosis Risk in Communities study. *Diabetes Care* 2006; 29: 2349–2354.

[238] Government of Canada SC. Projections of the Aboriginal Population and Households in Canada, 2011 to 2036. Available from: https://www150.statcan.gc.ca/n1/pub/91-552-x/91-552-x2015001-eng.htm (2015, accessed 11 September 2018).

[239] Si J, Yu C, Guo Y, et al. Season of birth and the risk of type 2 diabetes in adulthood: a prospective cohort study of 0.5 million Chinese adults. *Diabetologia* 2017; 60: 836–842.

[240] Vaiserman AM, Khalangot MD, Carstensen B, et al. Seasonality of birth in adult type 2 diabetic patients in three Ukrainian regions. *Diabetologia* 2009; 52: 2665–2667.

[241] Jongbloet PH, Van MS, Van E der V. Month-of-birth distribution of diabetics and ovopathy: a new aetiological view. *Diabetes Res Edinb Scotl* 1988; 9: 51–58.

[242] Jensen CB, Zimmermann E, Gamborg M, et al. No evidence of seasonality of birth in adult type 2 diabetes in Denmark. *Diabetologia* 2015; 58: 2045–2050.

[243] Telfer SJ. Fluoroquinolone antibiotics and type 2 diabetes mellitus. *Med Hypotheses* 2014; 83: 263–269.

[244] Dalton LM, Fhloinn N, M D, et al. Magnesium in pregnancy. *Nutr Rev* 2016; 74: 549–557.

[245] Dixon PH, van Mil SWC, Chambers J, et al. Contribution of variant alleles of ABCB11 to susceptibility to intrahepatic cholestasis of pregnancy. *Gut* 2009; 58: 537–544.

[246] Van Mil SWC, Milona A, Dixon PH, et al. Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. *Gastroenterology* 2007; 133: 507–516.

[247] Papacleovoulou G, Abu-Hayyeh S, Nikolopoulou E, et al. Maternal cholestasis during pregnancy programs metabolic disease in offspring. *J Clin Invest* 2013; 123: 3172–3181.

[248] Wesdorp I, Bosman D, de Graaff A, et al. Clinical presentations and predisposing factors of cholelithiasis and sludge in children. *J Pediatr Gastroenterol Nutr* 2000; 31: 411–417.

[249] Scott JD, Garland N. Chronic liver disease in Aboriginal North Americans. *World J Gastroenterol WJG* 2008; 14: 4607–4615.

[250] Chagnon P, Michaud J, Mitchell G, et al. A Missense Mutation (R565W) in Cirhin (FLJ14728) in North American Indian Childhood Cirrhosis. *Am J Hum Genet* 2002; 71: 1443–1449.

[251] Minuk GY, Pollock G, Uhanova J. Adult idiopathic cholestasis: a condition more common in the Canadian Inuit? *Int J Circumpolar Health*; 76. Epub ahead of print 15 October 2017. DOI: 10.1080/22423982.2017.1388104.

[252] Garner R, Guimond E, Senécal S. The Socio-Economic Characteristics of First Nation Teen Mothers. *Int Indig Policy J*; 4. Epub ahead of print 13 March 2013. DOI: 10.18584/iipj.2013.4.1.9.

[253] Stensballe LG, Simonsen J, Jensen SM, et al. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. *J Pediatr* 2013; 162: 832-838.e3.

[254] Roos LL, Nickel NC, Romano PS, et al. Administrative Databases. In: *Wiley StatsRef: Statistics Reference Online*. American Cancer Society, pp. 1–17.

[255] Spangler L, Chaudhari M, Barlow WE, et al. Using administrative data for epidemiological research: case study to identify persons with periodontitis. *Periodontol 2000*; 58: 143–152.

[256] Katz A, Chateau D, Bogdanovic B, et al. *Physician integrated network: a second look*, http://www.deslibris.ca/ID/244494 (2014, accessed 25 July 2018).

[257] Katz A. *A comparison of models of primary care delivery in Winnipeg*. Winnipeg: University of Manitoba. Manitoba Centre for Health Policy, 2016.

[258] Roos LL, Gupta S, Soodeen R-A, et al. Data quality in an information-rich environment: Canada as an example. *Can J Aging Rev Can Vieil* 2005; 24 Suppl 1: 153–170.

[259] Nickel NC, Martens PJ, Chateau D, et al. Have we left some behind? Trends in socio-economic inequalities in breastfeeding initiation: A population-based epidemiological surveillance study. *Can J Public Health* 2014; 105: 362–368.

[260] Nickel NC, Chateau DG, Martens PJ, et al. Data resource profile: Pathways to Health and Social Equity for Children (PATHS Equity for Children). *Int J Epidemiol* 2014; 43: 1438–1449.

[261] Mustard CA, Derksen S, Berthelot JM, et al. Assessing ecologic proxies for household income: a comparison of household and neighbourhood level income measures in the study of population health status. *Health Place* 1999; 5: 157–171.

[262] Janicke DM, Finney JW, Riley AW. Children's health care use: a prospective investigation of factors related to care-seeking. *Med Care* 2001; 39: 990–1001.