**Statistical Analysis Plan (SAP) for**

**Investigating Effect of Antibiotics Use on Asthma in Children**

**Version: 1.0**

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**1 INTRODUCTION**

In the context of the environmental factors affecting gut microbiome, antibiotics use is a prominent factor affecting the structure and maturity of the intestinal microbiota and subsequently the development of asthma.

Our collaborators at the BCCDC have investigated the association between antibiotics and asthma. At the population level, ecological trends were found showing attributable risks (Asthma) associated with reduction in Antibiotics use.

We plan to use CHILD birth cohort to explore whether similar observational trends can be replicated. By using the entire CHILD General Cohort and asthma diagnosis at different ages, we can increase sample size, the period of observation and adjust for covariates and confounders that an ecological study is unable to do by design.

Preliminary results in a subset of the CHILD study for which 16S rRNA of stool samples was done show an association between gut microbiome diversity and antibiotics use in the first three months of life. Further crude associations between antibiotics use and asthma revelead an increased risk. Taken together, the findings in CHILD would provide a plausible biological mechanism for the effect of antibiotics on immune-mediated phenotypic outcomes, while also offer the opportunity to test whether temporal trends of antibiotics use are associated with asthma risk.

**2 DATA SOURCE**

This project is aimed to investigate the association between exposure to antibiotics in early life and asthma in childhood using data from prospective Canadian Healthy Infant Longitudinal Development (CHILD) study.

CHILD study was launched in 2008 as a platform for defining the risk factors for asthma and allergy in Canadian children (childstudy.ca). This fully-recruited, population-based and finely phenotyped birth cohort of 3,495 infants in 4 provinces across Canada provides a unique national resource for transdisciplinary studies requiring longitudinal data on health, genetics, epigenetics and environ­mental exposures.

**3 ANALYSIS OBJECTIVES**

**Primary Objective**

To investigate the association between exposure to antibiotics within 1-year after birth and asthma in 5 years, with adjustment for confounding factors.

**Secondary Objectives**

1. To investigate the association between exposure to antibiotics within 1-year after birth and asthma in 3 years, with adjustment for confounding factors.
2. To investigate the association between exposure to antibiotics within 1-year after birth and Atopy + wheeze in 1 year, with adjustment for confounding factors.

**Exploratory Objectives**

1. To explore the effect of antibiotics usage at **different time point** on incidence of events (Atopy + wheeze at 1 year old, asthma at 3 and 5 years old), with adjustment for confounding factors.
2. To explore the **cumulative dosage** of antibiotics use on incidence of events, with adjustment for confounding factors.
3. To explore the **type of antibiotics** use on incidence of events, with adjustment for confounding factors.
4. To investigate the association between exposure to antibiotics and asthma, considering the **time of asthma** exposure and the **time of antibiotics** use.
5. To evaluate the association between antibiotics usage, gut microbiome and their interaction effect on events

**4 ENDPOINTS AND COVARIATES**

4.1 ENDPOINTS

**Asthma** is assessed using specialist-physician diagnosis, clinical questionnaire and parents’ self-reported information on asthma-like manifestation. Pooling all these sources of information, individuals are classified as definite, possible, or no asthma around their 3rd and 5th birthday visits. The time of asthma is defined as the diagnosis time by specialist/physician.

**Atopy and wheeze** are assessed separately. Atopic status is defined using skin prick tests at 1, 3, and 5 years of age. Wheeze is defined using both self-reported parents questionnaires and the CHILD study physician. Individuals are classified into the following categories: Control (i.e. asymptomatic), Atopy only, Wheeze only, and Atopy + Wheeze.

4.2 COVARIATES

**Antibiotics use** (dosage/type) of mother is recorded through questionnaire at pregnancy, birth, 3 month, 6 month and 12 month after birth. Breastfeeding time is also reported by mother through questionnaire. Antibiotics use (dosage/type) of child is recorded through questionnaire at birth, 3 month, 6 month and 12 month after birth.

**Antibiotics use within 1-year** is defined as any antibiotics usage of mother at pregnancy, at birth, during breastfeeding and any antibiotics use of child at birth, 3 month, 6 month and 12 month after birth. Age at first use of antibiotics is defined as the **time of first antibiotics use**.

To investigate the effect of antibiotic dosage, the **cumulative defined daily dosage** (DDD) is calculated. As recommended by the World Health Organization (WHO), cumulative DDD is used to quantify the cumulative dose of antibiotics, and the categories are “low dose”, “moderate dose”, and “high dose”.

The **type and antibiotics** are classified into xxx categories as

4.3 CONFOUNDERS

The potential confounders of asthma included race, sex, mode of delivery, having older sibling, weight of baby, parental health status asthma of parents, daycare attendance, breastfeeding and mother antibiotics use (potential route of exposure if breastfeeding) (**Table 1**)

**5 STATISTICAL METHODOLOGY**

5.1 STATISTICAL PROCEDURES

For the **primary objective**, a multivariable logistic regression will be used to establish the effect of antibiotics use within 1 year after birth on asthma at 5 years, with adjustment for confounders.

For secondary objectives, similar approaches will be applied predict atopy+wheeze at 1 year and asthma at 3 years.

**For exploratory objective 1 to 3,** multivariable logistic regressions will be used to predict events (incidence of Atopy + wheeze at 1 year old, asthma at 3 and 5 years old) using antibiotics usage, age of first use of antibiotics, DDD of antibiotics, and type of antibiotics, with adjustment for confounders.

**For exploratory objective 4,** multivariablecox regression model will be used to analyze the association between antibiotic exposure and events, with adjustment for confounders. Antibiotics exposure is a time varying predictor. Follow-up is censored at drop-out from the study, death or end of study period, whichever occurs first.

**For exploratory objective 5,**  permutational analysis of variance (phyloseq() package in R) is used to examine which antibiotics use metric (one time point vs. cumulative exposure) is associated with asthma at age 5 and age 3. Either a structural equation model will then be used to test the mediating effect of microbiome (first two axis of a PCA) on the association between antibiotics use on asthma. Alternatively we will use a multivariable cox regression model to analyze the association between antibiotic exposure and events including a microbial signature metric (either alpha diversity at 3 and 12 months, or 1st PCA axis at 3 and 12 months)

5.2 MISSING DATA

Every effort will be made to capture all required clinical data for all samples. Missing data will be considered missing completely at random (MCAR) and the individuals will be removed from the multivariate analyse.

**6 PROGRAMMING PLANS**

All statistical tests are two-sided using a 5% significance level and analyses will be performed in R version 3.5 (R Foundation, Vienna, Austria).

**8 APPENDICES**

Table 1 Potential Confounders of Asthma