# Protocol for Systematic Review & Meta-Analysis

**Title:** The association between early life antibiotic exposure and the gut resistome of young children: a systematic review and meta-analysis

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**Review question(s):**
How does early childhood antibiotic exposure affect the composition and diversity of the gut resistome?

**Searches:**

We used PubMed (MEDLINE), Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials to search for papers for inclusion. Additionally, we will also review references of selected papers to identify any additional papers for inclusion. Only peer reviewed papers that were published on or after January 1, 2000 will be included. This date restriction was included 1) due to the term “resistome” not being introduced in the antibiotic resistance literature before 2006 [Sampling the antibiotic resistome; D’Costa et al. *Science*. 2006:311] and 2) to help reduce potential for papers only exploring a subset of antibiotic resistance genes in any given sample.
Only papers written in English will be incorporated into this review due.

Initial search strategies for each database were drafted by RML with consultation from a Dartmouth College Biomedical Sciences Librarian. Briefly, the original search strategy included a combination of the exposure (e.g., antibiotic or specific type of antibiotic), outcome (e.g., resistance, resistome), population (e.g., children), and study system (e.g., gut) of interest. However, since our exposure is frequently intertwined in keywords associated with our outcome and we wanted to have a more conservative search strategy, we chose a search strategy that did not include keywords for antibiotics or a specific antibiotic for each database. Once the search strategy for all databases was drafted, it was peer reviewed by another Dartmouth College Biomedical Sciences Librarian and associated edits were incorporated.

**Types of studies to be included:**
Randomized controlled trials, cohort studies, case-control studies, ecological, quasi-experimental

**Additional exclusion criteria:**

Studies will be excluded if they:

- only assess antibiotic exposures given to mother (prior to birth or during delivery and labor)
- only include non-systemic antibiotic exposure (e.g., topical or eye drops)

- only compare an antibiotic exposed group to a probiotic exposed group
- have an unspecified population, exposure, or outcome
- assess antibiotic resistance genes only in non-human guts
- assess less than 10 antibiotic resistance genes
- do not use PCR methods, whole-genome sequencing, whole metagenome sequencing, or shotgun sequencing to assess antibiotic resistance genes
- are only interested in isolates from particular species (i.e., not a study of the resistome from all microbes in the gut)
- were published before 1/1/2000
- are not written in English
- are animal studies
- are reviews or commentaries (e.g., editorials, commentaries, systematic reviews)
- do not contain a comparison group (e.g., case series or case reports)
- do not demonstrate clear temporality that can demonstrate that the exposure preceded the outcome (e.g., cross-sectional and some ecological studies)
- are preprints
- only exist as conference abstracts

**Condition or domain being studied:** The gut resistome (collection of antibiotic resistance genes) of young children.

**Participants/population:** Infants and children under 5 years old

**Intervention(s)/exposure(s):** Systemic antibiotic exposure directly to the child before 5 years of age

**Comparator(s)/control:** No or less systemic antibiotic exposure during the study period

**Context:**

Infants and young children are exposed to high quantities of antibiotics compared to other age groups. As antibiotic use is tied to antibiotic resistance, understanding the unintended impacts of these medications are necessary. Previous research has revealed that the gut is a reservoir for antibiotic resistance genes, collectively referred to as the resistome. While prior systematic reviews have assessed the association between antibiotic use and bacterial composition of the microbiome, no systematic review has assessed antibiotic exposure beyond the neonatal period on antibiotic resistance genes in the gut of young children.

**Main outcome(s):**

The main outcomes of interest will assess the association between antibiotic exposure and antibiotic resistance genes (specifically the incidence/prevalence, abundance, and alpha diversity) in the gut of young children. We will prioritize assessing overall resistome load and alpha diversity. If possible, we will also assess the differences in exposure groups based on presence/absence of specific antibiotic resistance genes or

classes of antibiotic resistance depending on heterogeneity. These metrics were selected due to perceived likelihood of being identified in multiple studies and low possibility of differential misclassification. Whenever possible, we will assess these main outcomes in context to any antibiotic exposure and assess pre/post antibiotic exposure to control for intra- and inter-variation.

**Measures of effect:**

If appropriate, we will assess these main outcome(s) in a meta-analysis. For any analysis assessing abundance or diversity, mean difference between antibiotic exposure groups will be calculated. For presence/absence metrics, we intend to use risk or odds ratios as appropriate. If a meta-analysis is not appropriate, these main outcomes will be assessed qualitatively.

**Data extraction (selection and coding):**

Rayyan software [Rayyan – a web and mobile app for systematic reviews; Ouzzani et al. *Systematic Reviews*. 2016:5(120)] will be used to manage records. RML will perform the initial search and remove any duplicate entries. Titles and abstracts will be screened independently by 2 reviewers (RML and either DBK, JL, or HCW) for all papers identified using our search criteria. Full-text articles will be assessed for eligibility by 2 reviewers (RML and either DBK, JL, or HCW) independently. Data (see below) from eligible papers will be extracted by RML and at least one other author independently. For situations in which there are multiple reports from the same study, data will be extracted separately, and information will be combined afterwards. Steps in the screening phase will undergo a piloting phase as needed to enhance consistency across reviewers. RML will review all papers throughout the process to maintain consistency and reduce possible discrepancies between reviewers.

Any discrepancies or disagreements will be discussed between RML and the second reviewing author and, if no decision can be reached, will be resolved by AGH. Reviewers will not be blind to journal titles, study authors, or author affiliations.

The following data will be pulled (where available and applicable) from eligible studies using a standardized form which will be piloted before use:

- General information (title, author(s), year of publication, journal, research aims)
- Study design (RCT, cohort, etc.)
- Total number of participants
- Total number of participants by intervention
- Ages of participants at exposure and outcome measurements
- Study period
- Setting
- Inclusion/exclusion criteria
- Method of recruitment

- Antibiotic regimen [e.g., method of collection (medical records, interview), type, dose, duration, duration before sample collection, route of administration, reason for indication]
- Sequencing tools and platform used for antimicrobial resistance gene classification
- Antimicrobial resistance gene information (database used for classification, type, class of drug confers resistance for, relative abundance, length of duration in child, presence/absence)
- Outcome/risk measurements including: presence/absence of antibiotic resistance genes*, alpha diversity of the resistome, and resistance load
- Statistical tools & methods
- Other covariates considered (e.g., delivery mode, gestational age, maternal antibiotic exposure, siblings, pets, sex, feeding method, exposures to other medicines, socioeconomic status, etc.)
- Information for risk of bias assessment
- Comments

*****During the initial data extraction phase, only the individual antibiotic resistance gene name(s) and, if available, classes and any additional genetic identifiers will be pulled by the reviewers independently. As hundreds of genes could be identified and there may be inconsistent naming conventions, RML will take the compiled lists of genes probed across studies, standardize naming conventions using the Comprehensive Antibiotic Resistance Database [CARD 2020: antibiotic resistome surveillance with Comprehensive Antibiotic Resistance Database; Alcock et al. *Nucleic Acids Research*. 2020:48(D517-D525)], and then determine if additional analyses can be conducted based on guidelines discussed in the 'Strategy for data synthesis' section.

**Risk of bias (quality) assessment:**

Risk of bias assessments will be conducted by RML and a second author (DBK, JL, or HCW) independently. Risk of bias will be assessed separately for observational and randomized controlled trials. The Risk of Bias in Non-randomised Studies – of Interventions (ROBINS-I) [ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions; Sterne et al. *BMJ.* 2016:355(i4919)] will be used for observational studies. ROBINS-I will be used to assess study-level bias due to confounding, selection, intervention classification, deviations from intended intervention usage, missing data, measurement of outcomes, and selective reporting of results. For randomized controlled trials, RoB 2 will be used to assess study level bias due to randomization, deviation from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result [RoB 2: a revised tool for assessing risk of bias in randomized trails; Sterne et al. *BMJ.* 2019:366 (l4898)]. Results from these risk of bias assessments will be presented as a 'Risk of bias summary' figure using RevMan software [Review Manager (RevMan); The Cochrane Collaboration. 2020].

The strength of evidence for each outcome will be assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology which assesses risk of bias, consistency of the effect, imprecision, indirectness, and publication bias [The GRADE approach is reproducible in assessing the quality of evidence of quantitative evidence synthesis; Mustafa et al. *J Clin Epidemiol.* 2013;66].

If outcomes are identified in 10 or more studies, funnel plot analysis will be used to assess small study effects and possible meta-biases.

**Strategy for data synthesis:**

For the narrative overview, we will describe and compare our findings by antibiotic exposure group for both individual and overall resistome metrics. Descriptive plots such as bar plots or histograms may be used to demonstrate similarities or differences between studies. Summary of finding tables will be used to compare the exposure and outcomes of interest.

We will conduct a meta-analysis on outcomes of interest when at least 3 or more studies report comparable outcomes (e.g., report a count of unique antibiotic resistance genes or resistome Shannon index by antibiotic exposure group). We intend to prioritize analyses looking at antibiotic exposure vs. no exposure (as defined by each study). Normalization or standardization techniques may be used if metrics are consistent across papers but are reported differently.

Assuming there is a sufficient number of studies to complete a meta-analysis, the following outcomes will be assessed: mean difference in alpha diversity/ richness, mean difference in resistance load, and presence/absence of specific antimicrobial resistance genes or classes. Change scores for resistome metrics (i.e., calculations based on differences in resistome metrics pre and post antibiotic exposure) will be prioritized when possible. Forest plots with 95% confidence intervals will be used to display pooled results. Fixed and random-effects modelling will be considered with models selected based on heterogeneity testing. Accordingly, we will test for heterogeneity using the $X^2$ and $I^2$ tests.

Additional pooling methods and techniques may be employed. If data is unclear or incomplete but could be considered viable for inclusion in the meta-analysis, authors of the study may be contacted. Statistical analyses will be conducted in R [R: A language and environment for statistical computing; R Core Team. R Foundation for Statistical Computing; 2021.]

**Analysis of subgroups or subsets:**
Depending on the sample size for groups, subgroup analyses will be conducted. Subgroup analyses may assess how the exposure-outcome relationship varies based on possible confounders (e.g., gestational age, delivery mode, or diet), types of antibiotic resistance gene identification tool used (qPCR, whole-genome sequencing, shotgun sequencing), type or timing of antibiotic intervention(s), or geographic location.

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**Review team members and their organizational affiliations:**

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**Anticipated complete date:** October 2022

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**Conflicts of interest:** None noted.

**Language:** English

**Country:** United States of America

**Subject index terms:** Anti-Bacterial Agents; Resistome; Antibiotic Resistance; Microbiome; Children

**Additional comments:** The Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2020 [PRISMA 2020; Page et al. *BMJ*. 2021:372(71)] will be followed. This protocol was completed in conjunction with the PRISMA-P checklist [Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation; Shamseer et al. *BMJ*. 2015:349(g7647)]. This protocol in a similar form has also been uploaded to PROSPERO. The registration number will be added when available.