The association between early life antibiotic exposure and the gut resistome of young children: a systematic review

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ABSTRACT

Antimicrobial resistance is a growing public health burden, but little is known about the effects of antibiotic exposure on the gut resistome. As childhood (0–5 years) represents a sensitive window of microbiome development and a time of relatively high antibiotic use, the aims of this systematic review were to evaluate the effects of antibiotic exposure on the gut resistome of young children and identify knowledge gaps. We searched PubMed, Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials. A PICO framework was developed to determine eligibility criteria. Our main outcomes were the mean or median difference in overall resistance gene load and resistome alpha diversity by antibiotic exposure groups. Bias assessment was completed using RoB 2 and ROBINS-I with quality of evidence assessed via the GRADE criteria. From 4885 records identified, 14 studies (3 randomized controlled trials and 11 observational studies) were included in the qualitative review. Eight studies that included information on antibiotic exposure and overall resistance gene load reported no or positive associations. Inconsistent associations were identified for the nine studies that assessed resistome alpha diversity. We identified three main groups of studies based on study design, location, participants, antibiotic exposures, and indication for antibiotics. Overall, the quality of evidence for our main outcomes was rated low or very low, mainly due to potential bias from the selective reporting of results and confounding. We found evidence that antibiotic exposure is associated with changes to the overall gut resistance gene load of children and may influence the diversity of antimicrobial resistance genes. Given the overall quality of the studies, more research is needed to assess how antibiotics impact the resistome of other populations. Nonetheless, this evidence indicates that the gut resistome is worthwhile to consider for antibiotic prescribing practices.

Introduction

In 2019, antimicrobial resistance (AMR) was the twelfth leading cause of death globally with 1.27 million attributed deaths, making it a rapidly-growing problem. Many factors contribute to the increasing burden of AMR including the use and misuse of antibiotics in agriculture and humans. Many studies focus on phenotypic AMR using microbiological assays, but this ignores underlying antimicrobial resistance genes (ARGs) in human, animal, or environmental resistomes that can lead to antibiotic-resistant infections. Likewise, information on pathogenic antibiotic-resistant organisms is important, but ignores commensal and possibly pathogenic organisms that have the potential to transfer ARGs and provide colonization resistance to the gut microbiota. As there are many opportunities to mitigate these impacts in humans including preventing transmission and selection of ARGs through hospital and community spread, encouraging vaccination to decrease direct and indirect infection to resistant organisms, delaying antibiotic prescribing in favor of watchful waiting, and developing new microbiome-based therapeutics that can prevent the recurrence of antibiotic-resistant infections.
measuring the collection of all ARGs in microbial communities (resistomes) is crucial.

The largest reservoir for ARGs in humans is in the gut. Perturbations to the gut microbiome have been associated with numerous health concerns including loss of colonization resistance, an increase in potentially pathogenic antibiotic-resistant organisms, and reduced microbiome resiliency due to decreases in strain-level diversity. Systematic literature reviews have attempted to assess the human gut resistome, but persistent gaps remain, including our understanding of how perturbations lead to alterations in ARG composition and diversity.

As infants and young children are exposed to more antibiotics than any other age group, children represent an important subset of the population to understand the impact of antibiotics on their gut microbiota and ARGs. Predominant type and reason for antibiotic use vary by age and population characteristics but amoxicillin and azithromycin are typically identified as the most frequently used in young children. These antibiotics work through different mechanisms, but both have been associated with changes to the gut microbiome. Previous systematic literature reviews have explored the effect of antibiotics on the gut microbiome of children. Only one systematic review has assessed the gut resistome of children but this study only assessed the impact of antibiotics to the gut resistome of neonates. While understanding the impact of antibiotic exposure during the neonatal period is critical, the type, dose, duration, and indication for these antibiotic exposures is not representative of children beyond this period.

The objective of this systematic review was to assess how early childhood antibiotic exposure affects the composition and diversity of the gut resistome. In particular, we aimed to identify what is currently known about the association and what gaps persist in the literature. With the growing burden of AMR, this information can provide guidance on how to consider the resistome in antibiotic stewardship practices.

Methods

Protocol and registration

The protocol for this review was registered in advance to PROSPERO [CRD42021293328]. Any amendments to the protocol were noted in the Supplement. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines for reporting this review (see Supplement).

Eligibility criteria

Our eligibility criteria was based on our pre-specified Population, Intervention, Comparator, Outcome (PICO) framework. Specifically, we only included reports that focused on children under 5 years, had clear indication of direct systemic antibiotic exposure to the child, and included children that had a comparator or control group of children that had fewer (or zero) instances of antibiotic exposure. To ensure that our outcomes of interest could have been measured in the study, we only included studies that used non-culturable approaches to study antimicrobial resistance (e.g., whole metagenomic sequencing or qPCR). Additionally, we wanted the studies to truly be defined as resistome-wide studies so we required at least 10 ARGs to be profiled and excluded studies that only profiled phenotypic resistance in isolates. While the definition of the human gut resistome is fluid, a recent systematic review identified the minimum number of ARGs in a resistome study as 10. Reports with insufficient data on the exposure and outcomes of interest were excluded. Reports that did not directly report on the exposure and outcomes of interest but had sufficient individual-level metadata to assess both were included with re-analyzed data. We only considered randomized controlled trials and observational studies that (1) could clearly demonstrate that the exposure preceded the measurement of the resistome and (2) included a comparator group.

Information sources and search strategy

We searched for records in PubMed (MEDLINE), Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials. Only records that
were available on or after January 1, 2000 were included based on related systematic reviews using this cutoff.\textsuperscript{18,32} Likewise, “resistome” was not used in scientific literature before 2006,\textsuperscript{33} so, to reduce the number of studies only assessing a subset of ARGs in the resistome but not exclude resistome-type studies published before 2006, the cutoff of 2000 was used as a conservative restriction. Only papers written in English were included due to resource limitations. In addition to the general search, RML manually reviewed references of included reports for additional research articles to include. One research article was added per suggestion during peer review.

The search strategy for each database was drafted by RML with consultation and peer review by librarians from the Dartmouth Biomedical Libraries (see Supplement). Our search strategy included a combination of our population (e.g., infants and children), outcome (e.g., resistance and resistome), and study system (e.g., gut and stool). Since our exposure of interest (antibiotics) is frequently intertwined with keywords used for our outcome, we chose a more conservative search strategy that did not directly include a search of antibiotics.

**Data extraction and risk of bias assessment**

RML conducted the search of all databases on November 15, 2021 and extracted the information to Zotero to remove duplicates and records with any retraction notices.\textsuperscript{34} Entries were uploaded to Rayyan to manage data and remove any further duplicates.\textsuperscript{35} Rayyan is a web-based tool that enables independent screening and decision-making on record inclusion or exclusion. Two independent reviewers (RML and either DBK, JL, or HCW) completed all rounds of screening and eligibility assessment. Titles and abstracts were screened in Rayyan. Full-text of articles eligible for inclusion were uploaded to shared Zotero groups and independently reviewed for inclusion in Rayyan.

Data from all research articles were extracted separately by two independent reviewers (RML and either DBK, JL, or HCW) using a piloted standardized template (see Supplement) derived from The Cochrane Collaboration.\textsuperscript{36} Data extracted included general information on study design, total number of participants, ages of participants at the exposure and outcome measurement, setting, details on the exposure and outcome measurement, and covariate information. Information related to the bias assessment including method of recruitment and inclusion/exclusion criteria were also captured. Reports (e.g., abstracts, commentaries, and clinical trial information) from included studies were assessed in combination with research articles.

Risk of bias assessments were completed by RML and a second reviewer (DBK, JL, or HCW) independently in conjunction with the data extraction. Randomized controlled trial and observational study potential bias were assessed using different tools. Randomized controlled trials, including cluster-randomized trials, utilized risk-of-bias (RoB 2) to assess potential study-level bias due to randomization, deviation from intended interventions, missing outcome data, measurement of the outcome, and selective reporting of results.\textsuperscript{37} Observational studies were assessed using the Risk of Bias in Non-randomized Studies – of Interventions (ROBINS-I) tool, which assesses possible study-level bias due to confounding, selection bias, intervention classification, deviations from intended intervention usage, missing data, measurement of the outcome, and selective reporting of results.\textsuperscript{38} Some amendments to the ROBINS-I criteria were made to better capture biases inherent in resistome studies (see Supplement). Google Forms derived from the original bias assessment templates for each tool were utilized. After reviewing all articles, a joint meeting among RML, DBK, JL, and HCW was used to confirm relative bias assessment levels across both observational studies and randomized controlled trials. The robvis web platform was used to create stop-light figures.\textsuperscript{39}

For studies that had multiple research articles, data extraction, and risk of bias assessment were completed separately for each research article. If studies had multiple reports of the same outcomes from different time points before 5 years of life, all outcomes were reported in the Tables. Any discrepancies or disagreements were discussed between RML and the second reviewing author. In situations where no decision was reached, disagreements were resolved by AGH. None of the
reviewers were blinded to the journal titles, study authors, or author affiliations. All data extraction forms, bias assessments, instructions, and additional comments were available on the Google Drive shared with all authors.

**Main outcomes and synthesis measures**

Our primary outcomes of interest were overall gene resistance load and alpha diversity of the resistome with a secondary priority of extracting ARG presence and absence data. Mean or median difference between antibiotic exposure groups for both our primary metrics (as defined by each study) were the effect measures prioritized and studies were only included in summary of findings tables if one of these metrics could be derived. Overall resistance gene load was defined as the reported relative abundance of antimicrobial resistance genes in a given sample, but exact quantification varied by study (see Table 2). Richness was prioritized over other alpha diversity metrics post hoc due to the availability of richness data compared to other metrics of alpha diversity in studies. Any information about these measures, certainty around the estimate, and statistical significance were included. Associations between any type of systemic antibiotic and these outcomes were considered. In instances where re-analysis of the data was required to simultaneously measure the exposure and outcome of interest, RML re-analyzed the data using the following criteria:

1. If a study had sufficient individual-level antibiotic exposure and ARG data to assess overall resistance gene load and alpha diversity by antibiotic exposure group, re-analysis was performed on these datasets using R version 3.6.0.\(^{49}\) To assess the exposure and outcomes of interest, a fixed linear regression model adjusted for the day of sample collection was used. Only one stool sample per child was included in any re-analysis. Samples collected from the window directly following the study’s classification of the antibiotic exposure window were prioritized to best reflect the direct effects of antibiotic exposure.

2. If either of these outcome variables were available by antibiotic exposure groups but had to be extracted from data tables, data from these tables was summed or combined to assess the outcome by group.

3. If either of these outcome variables were available by antibiotic exposure groups but had to be extracted from Figures, data extraction from Figures was completed using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/).

All re-analysis of data from individual studies was documented in Table S1 and in the accompanying R code. Since we had a reduced level of granularity and significant heterogeneity across studies, we were not able to perform a formal meta-analysis on these data per protocol.

**Quality of evidence**

Strength of evidence for each outcome was assessed based on the main components of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology, which assesses risk of bias, consistency of the effect, imprecision, indirectness, and other bias (see Supplement).\(^{50}\) Since this systematic review consisted of a mixture of randomized controlled trials and observational studies, the quality of evidence for studies started as Low per GRADE protocol but different factors were used to downgrade or upgrade the quality of evidence.\(^{51}\)

**Results**

**Overview of included studies**

From our initial search, we identified 4877 records and assessed the full-texts of 119 for eligibility (Figure 1). Additionally, we assessed seven records as independent research articles via reference searching of full-text articles that were included. A research article was added during the peer review process that was not published during our initial search. Reports were most often excluded due to: a lack of child antibiotic exposure or comparison group (n = 28), outcomes of interest not reported (n = 22) or did not meet our eligibility requirements for the participant population for this review (n = 15). Information regarding each full-text
### Table 1. Descriptive overview of studies identified.

| Study ID | Aim | Study design | Country | Population description | Number of eligible participants | Age of participants at exposure measurement | Antibiotic exposed participants; No. | Antibiotic unexposed participants; No. | Reason for antibiotic exposures | Administration of antibiotic exposures | Reporting of antibiotic exposures | Comments |
|----------|-----|--------------|---------|------------------------|---------------------------------|------------------------------------------|----------------------------------------|----------------------------------------|---------------------------------|----------------------------------------|---------------------------------|----------|
| ARMCA Study26-34 | Evaluate the effect of 3 different antibiotics vs. placebo on the gut resistome of children under 5 | Randomized controlled trial | Burkina Faso | Children from 2 rural communities in Nouna District | 120 | 6-59 months of age | Amoxicillin, azithromycin, or cotrimoxazole exposed to 5 days of treatment; 31 in each group randomized; 29, 31, 30 respectively with samples collected | Placebo given for 5 days of treatment; 31 randomized, 30 with samples collected | Prophylactic use to prevent morbidity and mortality | Pediatric oral suspension | Directly observed |
| D’Souza 202035-37 | Analyze differences in microbiome characteristics among HIV exposed, uninfected infants by cotrimoxazole exposure | Randomized controlled trial | South Africa | HIV-exposed, uninfected infants | 63 | 6 weeks to 6 months of age | Cotrimoxazole exposed once daily during study period; 34 | Cotrimoxazole unexposed; 29 | Prophylactic use to prevent HIV | Oral | Self-report by mothers at each study visit |
| MORDOR Study41-43,47-49 | Assess the effects of bi-annual mass azithromycin treatments on gut resistome of preschool children | Cluster-randomized trial | Niger | Randomly sampled preschool children from villages in Logos and Boboye departments | 30 communities | 1-59 months of age | Mass azithromycin administered biannually; 15 villages | Mass placebo administered biannually; 15 villages | Reduce mortality | Oral | Directly observed | Outcome measurements taken at: Baseline41,43,46,57 24 months41,42,48 36 months41,42,48 48 months41,42,48, 60 months41 |

### Observational studies

| Study ID | Aim | Study design | Country | Population description | Number of participants | Age of participants | Antibiotic exposure | No antibiotic exposure | Variable in infants under 4 months, mostly for bacterial infections. For antibiotics given in the 4-12 month window, mainly for upper airway and middle ear infections | Mainly orally | Medical records and national prescription registers | 98 total infants were included in the study with metagenomics samples, but only the 83 vaginally delivered infants had resistome information available |
|----------|-----|--------------|---------|------------------------|-----------------------|-------------------|-----------------------|------------------------|---------------------------------|------------------|---------------------------------|------------------|
| Badhed 201541 | Conduct a metagenomic analysis of mother-infant pairs in the first year of life to assess the impact of delivery mode and feeding | Longitudinal cohort | Sweden | Full-term vaginally delivered infants in Halland | 83 | Up to 1 year | Antibiotic exposure 0-4 months; 3-4-12 months; 18 Ever exposed | No antibiotic exposure 0-4 months; 80-12-12 months; Never exposed; 62 | Variably in infants under 4 months, mostly for bacterial infections. For antibiotics given in the 4-12 month window, mainly for upper airway and middle ear infections | Mainly orally | Medical records and national prescription registers | 98 total infants were included in the study with metagenomics samples, but only the 83 vaginally delivered infants had resistome information available |
| Study                                                                 | Design, Setting                                                                 | Participants                                                                 | Control group                                                                                          | Exposure                                                                                     | Outcomes                                                                                   | Exposure-related complications | Comparisons                                                                                      |
|----------------------------------------------------------------------|---------------------------------------------------------------------------------|------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------|---------------------------------------------------------------------------------------------|
| Eiasson et al., 2018                                                 | Prospective cohort study, Norway                                                 | Premature infants from 6 NICUs                                              |                                                                                                       | After first week to 4 months; Broad antibiotic exposure at 28 days; Narrow antibiotic exposure at 28 days; Complications related to preterm birth ** | Intravenous** Medical records ** Some infants in this set were exposed to probiotics       |                                 |                                                                                             |
| Houigian et al., 2018                                                | Prospective cohort study, United States                                         | Infants in NICU within Inova Fairfax Hospital in Falls Church, Virginia     |                                                                                                       | Up to discharge from NICU; Antibiotic exposure ever; No antibiotic use ever; 12                   | Intravenous** Collected while in the NICU Antibiotic exposure differed by NICU with infants receiving more antibiotics in the shared room (traditional) NICU compared to the private room (new) NICU |
| Li et al., 2021                                                      | Prospective cohort study, Denmark                                               | Infants drawn from Copenhagen Environmental Studies on Asthma in Childhood 2010 birth cohort |                                                                                                       | Under 1 year; Antibiotic usage in first year of life; 311                                       | Not specified Measured during clinic visits and validated against registry records            |                                 |                                                                                             |
| Neontal Microbiome and Necrotizing Enterocolitis Study or St Louis Neonatal Microbe Initiative at Washington University | Densely sample the gut microbiota and resistome of preterm and full-term infants exposed and unexposed to antibiotics | Preterm infants seen in the NICU at the St. Louis Children's Hospital and healthy term infants from the Neonatal Microbiome and Necrotizing Enterocolitis Study or St. Louis Neonatal Microbiome Initiative |                                                                                                       | 8 months; Birth to hospital leave (maximum of 91 days); Up to 21 months                          | Not amoxicillin exposed for 10 days; Early antibiotic exposure only; Term antibiotic naïve infants; 17 | Not specified | Predominantly intravenously Parental questions onnaire and verified via the infants’ medical records |

(Continued)
| Study                                                                 | Design            | Study population                                                                 | Study population (Continued)                                                                 | Number of participants | Exposed to antibiotics | Infants not exposed to antibiotics | Clinical problems at birth | Antimicrobial therapy | Other remarks |
|----------------------------------------------------------------------|-------------------|----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------|-------------------------|----------------------------------|--------------------------|----------------------|--------------|
| Rahman 2018** admitted premature infants to NICU, Magee-Women’s Hospital | Longitudinal cohort | United States, Magee-Women’s Hospital in Pittsburgh, Pennsylvania                | Premature infants with seONS from NICU at Magee-Women’s Hospital in Pittsburgh, Pennsylvania | 107                    | First week to 3 months | Received antibiotics after the first week of life; 36 | Varies but includes late-onset sepsis, NEC, or another disease | Intravenous**            | Medical records** |
| Reymann 2022** admitted premature infants with seONS from NICU         | Longitudinal study | Netherlands, Magee-Women’s Hospital in Pittsburgh, Pennsylvania                  | Infants with seONS from NICU in multiple hospitals                                         | 225                    | First week of life    | Exposed to antibiotics of amoxicillin and cefotaxime, co-amoxiclav and gentamicin, or penicillin and gentamicin; 47, 49, and 49 infants in each respective group | Not exposed to antibiotics in first week of life; 90 | seONS                 | Intravenous   |
| Rose 2017** investigated the resistome of 11 healthy premature infants | Longitudinal cohort | United Kingdom, Healthy, premature infants in NICU of Imperial College Healthcare Service Trust in London | Healthy, premature infants in NICU of Imperial College Healthcare Service Trust in London | 11                     | Until initial hospital discharge (max: 43 days of life) | Exposed to course 1 antibiotics; 8 Exposed to course 2 antibiotics; 8 | Not exposed to course 1 antibiotics; 3 Not exposed to course 2 antibiotics; 7 | Prophylactic antibiotic treatment related to premature birth | Intravenous** | Medical records** | Study randomizes antibiotic exposure types, but not antibiotic exposure vs. no exposure (i.e., our main exposure of interest). This led to our decision to classify this study as observational for the purposes of this systematic review. |
| Thanert 2021 | Evaluate the effects of antibiotic-driven intestinal dysbiosis in gut microbiota and resistome of patients with surgically-induced short bowel syndrome (SBS) compared to preterm and term controls |
|-------------|--------------------------------------------------------------------------------------------------------|
| Patients with surgically-induced SBS from St. Louis Children's Hospital | 19 | Up to 4 years | Current antibiotic use; 5* Antibiotic use in past month; 11* Not current antibiotic use; 14* No antibiotic usage in past month; 8* | Non-SBS controls were selected from the Neonatal Microbiome and Necrotizing Enterocolitis Study or St Louis Neonatal Microbiome Initiative at Washington University |

| Yassour 2016 | Conduct a natural history study of the gut microbiome comparing children exposed to 9 or more antibiotics versus unexposed |
|-------------|--------------------------------------------------------------------------------------------------------|
| Children in the DIABIMMUNE Study from Espoo | 39 | Before 3 years | Received 9 or more antibiotics in first 3 years of life; 20 No antibiotic exposure in first 3 years of life; 19 Mainly to treat otitis media and respiratory infections Systemic, orally treated | Reported by parents prospectively in study diary* |

*Eligible participants indicates children under 5 with both antibiotic exposure and resistome information
**Indicates assumed value based on population characteristics (e.g., infants in NICU almost always given antibiotics intravenously)
Figure 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers, and other sources.

report’s inclusion or exclusion is available in Table S2. Ultimately, 25 reports were included in this systematic review spanning 14 independent studies (Table 1).

Of the 14 independent studies, three40–42,52,55,57,58 were randomized controlled trials and 1114,15,43–48,59–67 were observational studies. The sample sizes across studies varied widely, with many reports taking repeated samples from the same participants.14,15,40,43–48,60,61,63,64 There were several reports14,43,45,46,48,60,61,66 that had less than 50 participants with eligible resistome data with only a few studies capturing resistome data from over 100 participants.41,42,44,52,57,58,64 All the studies considered beta-lactam antibiotics except for the MORDOR Study41,42,57,58 which focused on antibiotic exposure to azithromycin and a study focused on evaluating the adverse effects of cotrimoxazole among HIV-exposed uninfected infants.40,55 All studies utilized short-read sequencing technology (e.g., Illumina HiSeq or NextSeq) or qPCR as opposed to long-read sequencing (e.g., HiFi sequencing). Different ARG databases for shotgun metagenomics were used across studies with CARD the most frequently used14,15,40,43–46,63 followed, respectively, by MEGARes,41,42,52,57,58,64 Resfams,14,47,63 ARG-ANNOT,66 ARDB,59 CosmosID,60 PARFuMS,61 and the NCBI AMR database.48

Over all resistance gene load
We identified eight studies14,40–48 with available mean or median overall resistance gene load by antibiotic exposure group data. Consistently, these studies found that there was either no difference or a statistically significant increase in overall resistance gene load between children classified as antibiotic exposed versus unexposed (Table 2). Children in the MORDOR Study were exposed to bi-annual azithromycin or placebo and followed for 60 months.41,42,57,58 While the MORDOR Study did not report overall resistance gene load outcomes, at both the 36 and 48 month time point, multiple resistance determinant (defined based on having a gene fraction greater than 80%)42 classes other than macrolides determinants were increased including beta-lactams and sulfonamides.42 Likewise, at these time points, all the mean resistance loads for each determinant, even if not statistically significantly different between groups, were increased in the azithromycin-exposed group.42 However, by 60 months there was no
evidence that non-macrolide resistance determinants were different between groups. In total, these data suggest that antibiotic exposure is capable of causing collateral changes to the resistome, but these changes may only occur in a subset of children and may vary by frequency and timing of exposures. Considering these factors, we gave this outcome a Low quality of evidence value (see Supplement).

**Alpha diversity of ARGs**

There were nine studies\(^\text{14,40,44,47,48,52,59,64,66}\) that assessed alpha diversity or richness by antibiotic exposure (Table 3).

The two randomized controlled trials that evaluated richness identified children exposed to amoxicillin\(^\text{52}\) or cotrimoxazole\(^\text{40,52}\) both had statistically significant increases in the number of unique ARGs in antibiotic treated children compared to the unexposed or placebo group. In contrast, observational studies in populations of generally healthy infants born predominantly at term exposed to greater quantities of antibiotics had a statistically significant but only slight increase in ARG richness at 1 month,\(^\text{64}\) approximately the same number of resistance gene types,\(^\text{44,59,64}\) or a decreased richness of ARGs.\(^\text{14}\) While these results are discrepant, they are likely a reflection of the underlying population characteristics and microbial composition. A “central dogma” of the gut microbiome field is that participants that have a higher microbial alpha diversity or greater strain variation are better able to respond to perturbations.\(^\text{16}\) Since the resistome is interconnected with the gut microbiome,\(^\text{14,44,68}\) results for resistome alpha diversity are likely related. For instance, in Gasparrini et al.\(^\text{14}\) out of 54 metagenomes with high resistance loads, 41 (76%) were dominated by a single species. *Escherichia coli* was the most frequently identified dominant species, but other dominant species included *Enterococcus faecalis*, and *Klebsiella pneumoniae*. They found alpha diversity was statistically significantly lower among children with early and subsequent antibiotic exposure compared to near-term children unexposed to antibiotics. Meanwhile, Li et al.\(^\text{44}\) also found that *E. coli* abundance contributed to the resistome profile, but at a smaller abundance per sample. No difference in ARG richness was noted between exposed and unexposed infants. Thus, not only which microbes are present, but their relative abundance in a given population is likely to be a main driver of the heterogeneous findings.

**Studies separated into 3 groups**

While there was much heterogeneity across studies included, we identified three main categories defined by study design, participants, country, and antibiotic exposure (Table 4). These groups were used to summarize our findings for outcomes of interest and identify gaps in the literature.

The studies in the first group\(^\text{40–42,52,57,58}\) were randomized controlled trials that had the aim of assessing whether prophylactic antibiotic exposure to prevent infection or reduce malnutrition affects the gut resistome. Two of the studies were focused at the individual level,\(^\text{40,52,55}\) while the unit of analysis for the MORDOR Study was at the *grappe* or village level.\(^\text{41,42,57,58}\) Across these studies, there was evidence suggesting that azithromycin or cotrimoxazole exposure was associated with increased prevalence of associated ARGs. In the ARMCA Study,\(^\text{52–54}\) macrolide ARG prevalence was 87.1% in children exposed to azithromycin compared to 33.3% in the control group. Similarly, macrolide ARG prevalence was 68.0% in communities exposed to azithromycin compared to 46.7% in the placebo-controlled communities at 24 months in the MORDOR Study.\(^\text{57}\) For cotrimoxazole, in the ARMCA Study,\(^\text{52–54}\) the risk ratios comparing exposed and unexposed children for both sulfonamide and trimethoprim resistance genes were significant [sulfonamide: 8.83 (95% CI: 1.01–77.0) and trimethoprim: 3.29 (95% CI: 1.08 to 9.95)]. Similarly, *dfr* and *sul* resistance gene richness for exposed infants was greater than in unexposed infants (mixed effects linear regression *p*-value = 0.016). The studies also support the notion that antibiotics affect ARGs beyond those conferred by the antibiotic but likely not to the same extent. Specifically, in the MORDOR Study, they found that beta-lactam resistance gene determinants were increased by a factor difference of 2.13 (95% CI: 1.33–4.02) and 1.98 (95% CI: 1.10–4.57) at 36 and 48 months\(^\text{42}\) respectively but did not differ by exposure group at 60 months.\(^\text{41}\) Neither the prevalence of
Table 2. Association between antibiotic exposure and overall resistance gene load.

| Study ID | Antibiotic Exposure Group (N) | Comparison (N) | Age of participants at outcome measurement | ARG database used | Unexposed or Control group | Intervention group | Difference, Percentage change | Statistical Significance | Comments |
|----------|--------------------------------|----------------|--------------------------------------------|------------------|-----------------------------|-------------------|-------------------------------|------------------------|----------|
| **Randomized controlled trials** |
| D’Souza 2020* 45 | Once daily cotrimoxazole exposure during study period (N = 34) | No cotrimoxazole exposure during study period (N = 29) | 4 months, 6 months | CARD | Median or mean log-transformed resistance gene abundance | Median: 4 months: 7.2, 6 months: 7.1 | Median: 4 months: 8.0, 6 months: 8.0 | Mean difference: 4 months: 0.71 (95% bootstrapped CI: 0.2–1.2), 6 months: 0.85 (0.1–1.7) | P-value: 4 months = 0.037, 6 months = 0.095 (Wilcoxon test) | Median values were estimated from Figure with mean difference and p-value reported in paper |
| MORDOR Study 41,42 | Community exposed to bi-annual azithromycin (N = 14 communities) | Community exposed to bi-annual placebo (N = 15 communities) | 36, 48, 60 months | MEGARes | Mean normalized abundance of resistance determinants (reads/million) | 36 months: 951.4, 48 months: 868.4, 60 months: 699.4 | 36 months: 1930.6, 48 months: 1661.1, 60 months: 1025.3 | 36 months: +979.3, +102.9, +48 months: +792.6, +91.3, 60 months: +325.9, +46.6% | Significance not calculable for individual children |
| **Observational studies** |
| Esaissen 2018 43 | Broad antibiotic exposure after first week among all preterm infants (N at 28 days = 7, N at 4 months = 9) | Narrow antibiotic exposure beyond first week of life (N at 28 days = 15, N at 4 months = 15) | 28 days, 4 months | CARD | Mean absolute counts/total abundance | 28 days: 16.8, 4 months: 23.2 | 28 days: 125.2, 4 months: 36.3 | 28 days: +108.4, +645%, 4 months: +13.1, +56% | Statistically significant at 28 days, not significant at 4 months | Estimates derived from table, significance reported in paper |
| Li X. 2021 44 | Any antibiotic exposure in first year of life (N = 311) | No antibiotic exposure in first year (N = 351) | 1 year | CARD | Mean of summed ARG abundance | 1223 | 1377 | +154, +12.6% | P-value = 0.078 (Wilcoxon test) | No CI or SD given |
| Neonatal Microbiome and Necrotizing Enterocolitis Study or St Louis Neonatal Microbiome Initiative at Washington University 14* | Children with early and subsequent antibiotic exposure (N = 32) | Children born near term and antibiotic naïve (N = 17) | Up to 21 months of age | CARD | Median RPKM | 1681 | 1971 | +289.9, +17.2% | P-value < 0.05 (Wilcoxon test) | Multiple samples per child were included per Figure |

(Continued)
| Study ID | Antibiotic Exposure Group (N) | Comparison (N) | Age of participants at outcome measurement | ARG database used | Metric used | Unexposed or Control group | Intervention group | Difference, Percentage change | Statistical Significance | Comments |
|----------|-------------------------------|----------------|---------------------------------------------|------------------|-------------|---------------------------|------------------|-----------------------------|----------------------|----------|
| Oulu, Finland Study Tapiainen 2019 45* | Infants exposed to intrapartum and postnatal antibiotics during first week of life (N = 15) (N = 8) | Infants unexposed to intrapartum and postnatal antibiotics during first week of life (N = 10) (N = 8) | 3–7 days of life | CARD | Mean AMR gene copies per sample | 7560 | 9850 | +2290,+30.3% | P-value = 0.16 (two tailed t-test) |          |
| Li W. 2021 46* | Antibiotic exposure after the first week (N = 36) | No antibiotic exposure after first week of life (N = 71) | 12 months | CARD | Mean copies per million | 1656.3 | 1757.8 | +101,+6.1% | P-value = 0.93 (two tailed t-test) |          |
| Rahman 2018 47** | Antibiotic use in the past month (N = 10) | No antibiotic use in past month (N = 9) | 0–4 years | CARD and NCBI AMR database | Linear regression model adjusted for day of sampling | 4234 | 6270.6 | +2036.6, +48.1% | P-value = 0.51 (linear regression) | Earliest sample point per child included based on exposure window |
| Thanert 2021 48** | Infants exposed to intrapartum and postnatal antibiotics during first week of life (N = 15) (N = 8) | Infants unexposed to intrapartum and postnatal antibiotics during first week of life (N = 10) (N = 8) | 3–7 days of life | CARD | Mean AMR gene copies per sample | 7560 | 9850 | +2290,+30.3% | P-value = 0.16 (two tailed t-test) |          |

Notes: *Approximation based on values in a bar plot or box and whisker plot. **Individual-level re-analysis was conducted on the data.
Table 3. Association between antibiotic exposure and resistome alpha diversity.

| Study ID          | Antibiotic Exposure Group | Comparison                                                                 | Age of participants at outcome measurement | ARG database used | Metric used | Unexposed or Intervention group | Difference, Percentage change | Significance | Comments |
|-------------------|---------------------------|----------------------------------------------------------------------------|--------------------------------------------|-------------------|------------|-------------------------------|-------------------------------|--------------|----------|
| **Randomized controlled trials** |                           |                                                                            |                                            |                   |            |                               |                               |              |          |
| ARMCA Study 52-54 | 5 day course of amoxicillin (N = 30) | 5 day course of placebo (N = 30)                                           | 6–59 months of age                         | MEGAREs           | Mean Chao 1 richness          | 23.9                          | Amoxicillin: +18.7, +78.2%     | P-value amoxicillin = 0.02 azithromycin = 0.15 cotrimoxazole = 0.049 |           |          |
| D’Souza 2020      | Once daily cotrimoxazole exposure during study period (N = 34)            | No cotrimoxazole exposure during study period (N = 29)                      | 4 months, 6 months                         | CARD              | Median richness               | 4 months: +21.3, +51.5%       | P-value = 0.005 (mixed effects model estimate) | Significance value derived from mixed effects model in D’Souza 2020 that adjusted for time, read count, and a random intercept for infant |           |          |
| **Observational studies** |                           |                                                                            |                                            |                   |            |                               |                               |              |          |
| Bäckhed 2015      | Antibiotics after 4 months (N = 18)                                      | No antibiotics after 4 months (N = 65)                                      | 12 months                                  | ARDB             | Mean number of resistance types | 12 months: +2.7, +29.6%       | P-value = NS (Wilcoxon test) | Significance reported in manuscript |           |          |
| Li X. 2021        | Any antibiotic exposure in first year of life (N = 311)                   | No antibiotic exposure in first year (N = 351)                             | 1 year                                     | CARD             | Median richness               | 55                             | P-value = 0.5 (one-way ANOVA)  | Multiple samples per child were included |           |          |
| Neonatal Microbiome and Necrotizing Enterocolitis Study or St Louis Neonatal Microbiome Initiative at Washington University | Antibiotic exposure after early and subsequent antibiotic exposure (N = 32) | Up to 21 months of age                                                      | Up to 21 months of age                     | CARD             | Median richness               | 97.3                           | P-value < 0.05 (Wilcoxon test) |                       |           |          |
| Rahman 2018       | Antibiotic exposure after the first week of life (N = 36)                 | No antibiotic exposure after first week of life (N = 71)                    | Up to 86 days                              | Resfams           | Linear regression model       | 32.3                           | P-value = 0.09 (linear regression) | Last sample collected from infant to account for exposure window |           |          |

(Continued)
| Study ID     | Antibiotic Exposure Group | Comparison                                      | Age of participants at outcome measurement | ARG database used | Metric used                      | Unexposed or Control group | Intervention group | Difference, Percentage change | Significance | Comments                                                                                         |
|-------------|---------------------------|-------------------------------------------------|--------------------------------------------|-------------------|----------------------------------|---------------------------|-------------------|---------------------------------|-------------|---------------------------------------------------------------------------------------------|
| Reyman 2022 | Antibiotic exposure for sEONS in first week of life (N = 147) | No antibiotic exposure for sEONS in first week of life (N = 78) | 1 month | (qPCR Fluidigm primers used; some match reported organisms from the Antibiotic Resistance Database) | Median observed number (richness) | 7.5 | 9 | +1.5, +20% | P-value = 0.02 (Wilcoxon test) | AMR gene diversity did not differ by antibiotic status in cross-sectional analysis at other ages or in a temporal analysis over the first year of life |
| Rose 2017   | Infants exposed to one or two courses (N = 9) | Infants unexposed (N = 2) | Up to 43 days of life | ARG-ANNOT | Linear regression model adjusted for day of sampling | 9.2 | 5.9 | −3.3, −35.8% | P-value = 0.45 (linear regression) |
| Thanert 2021 | Antibiotic use in the past month (N = 10) | No antibiotic use in past month (N = 9) | 0–4 years | CARD and NCBI AMR database | Linear regression model adjusted for day of sampling | 37.8 | 45.2 | +7.4, +19.5% | P-value = 0.59 (linear regression) | Earliest sample point per child included based on exposure window |

Notes: *Approximation based on values in a bar plot or box and whisker plot. **Individual-level re-analysis was conducted on the data.
Table 4. The effect of antibiotic exposures by different study groups.

| Group | Study design | Participants | Country | Antibiotic Exposures | Research question | Key Findings |
|-------|--------------|--------------|---------|----------------------|------------------|--------------|
| Group 1 | Randomized controlled trials | Children <5 years old in communities with a high burden of infection and malnutrition | Niger, South Africa, Burkina Faso | Macrolides (azithromycin), sulfanilamides (cotrimoxazole), beta-lactams (amoxicillin) | How does prophylactic antibiotic use to prevent morbidity and mortality impact the gut resistome? | Consistent evidence of increased abundance and diversity of ARGs conferring resistance to antibiotic given with some evidence suggesting increases to other ARGs. |
| Group 2 | Longitudinal, observational studies | Generally healthy populations of children <3 years old | Finland, Sweden, Denmark, Netherlands, USA | Variety of antibiotics including: beta-lactams, aminoglycosides, sulfanilamides, macrolides | How does typical antibiotic use for common childhood ailments impact gut resistome? | Studies in this category suggest that overall resistance gene load and ARG alpha diversity are not significantly impacted by antibiotics, but specific ARGs do increase after exposure to antibiotics. |
| Group 3 | Longitudinal, observational studies of children selected based on prematurity or NICU exposure | Predominantly children <2 years old born preterm or infants in NICU | Norway, USA, UK | Greatest variety of antibiotics including: beta-lactams, aminoglycosides, vancomycin, clindamycin, sulfanilamides, macrolides | How does antibiotic use during early-life hospitalization impact the gut resistome? | The microbiome is less resilient and most likely to be affected by perturbation. This leads to the highest levels of resistome heterogeneity between studies. |
these resistance determinants nor other non-macrolide determinants differed at 24 months.\textsuperscript{57,58} Similarly, none of the antibiotics assessed in the ARMCA Study\textsuperscript{52–54} resulted in a prevalence difference in beta-lactam resistance genes but both amoxicillin and azithromycin exposure were associated with increased prevalence of sulfonamide resistance genes [risk ratio for amoxicillin: 15.3 (95% CI: 1.80–129.1) and azithromycin: 16.0 (95% CI 1.91–133.5)] in children 6–59 months of age.

The second group\textsuperscript{15,44–46,59,61,64} assessed the effects of antibiotic exposures primarily for common infections, such as clinical concerns requiring antibiotics in the first week of life,\textsuperscript{45,46,64} respiratory illnesses,\textsuperscript{15,59} and otitis media\textsuperscript{15,59} afflicting children under 3. All but one\textsuperscript{61} of the reports were jointly interested in the impacts of multiple early life factors in addition to direct antibiotic exposure including delivery method,\textsuperscript{15,44,59,64} maternal antibiotic exposures,\textsuperscript{44–46,59,64} the child’s diet,\textsuperscript{15,44,59,64} and other environmental exposures.\textsuperscript{44,64} Multiple studies also profiled the maternal microbiome with the goal of understanding if antibiotic exposure modifies vertical transfer.

![Figure 2](image.png)

**Figure 2.** Assessment of potential bias sources for included a) randomized controlled trials and b) observational studies. Randomized controlled trials were assessed using the RoB 2 tool while observational studies were assessed using ROBINS-I.
of microbes or ARGs.\textsuperscript{45,46,59,61} All studies included infants up to 1 year of age and only one study had information on children 1–3 years of age.\textsuperscript{15} Compared to studies from the other groups, we found evidence that the resistome of children in this group is less sensitive to the effects of antibiotic exposure. These main findings are discussed in Yassour et al.\textsuperscript{15} and Moore et al.\textsuperscript{61} and is evidenced by the null effects of antibiotic exposure on overall resistance gene load and alpha diversity as noted in other studies.\textsuperscript{44–46,59} However, while overall resistome outcomes may only be moderately impacted by antibiotic exposure, antibiotic exposure can still impact individual ARGs via alterations to microbial composition. As identified in Yassour et al.\textsuperscript{15} certain ARGs on chromosomes may peak in abundance directly after antibiotic exposure and then decline, while some ARGs on mobile genetic elements persist long after antibiotic exposure. The authors found that the abundance changes of ARGs on chromosomes was correlated to the abundance of species, such as \textit{E. coli}, \textit{Klebsiella pneumoniae}, and \textit{Ruminococcus gnavus}. This agrees with Li X. et al.\textsuperscript{14} which found \textit{E. coli} to describe the resistome pattern and the larger literature\textsuperscript{68–70} suggesting \textit{E. coli} abundance has a major influence on ARG abundance in infants from generally healthy children cohorts.

The last group of studies\textsuperscript{14,43,47,60,62,63,66} had the broad aim of understanding how early life antibiotic exposures impact the gut resistome of children born prematurely or with extended exposure to the Neonatal Intensive Care Unit (NICU). Only Gasparrini et al.\textsuperscript{14} evaluated children beyond 4 months with the focus primarily on antibiotic exposures occurring during early life hospitalization. This was also the only study to compare the resistome of preterm infants to near-term infants.\textsuperscript{14} Hourigan et al.\textsuperscript{60} attempted to separate the effects of hospitalization and antibiotic exposure, but identified that both components impacted the abundance of ARGs. Compared to studies in Group 2, the children in Group 3 studies have resistome compositions more sensitive to antibiotic exposure. The overall resistance gene load was increased\textsuperscript{14,43,47} in children exposed to any or multiple antibiotic exposures compared to unexposed infants with specific ARGs differentially abundant in antibiotic-exposed children.\textsuperscript{60,62,63} Interestingly, studies in this set looking at richness\textsuperscript{14,47,66} found an inverse association between antibiotic exposure and ARG richness. Similarly to studies in other groups, changes to gut microbiota impacted the resistome\textsuperscript{14,47,63,66} with multiple studies noting that species dominance (i.e., the species comprises >50% of the sample) was an important factor in resistome composition.\textsuperscript{14,63,66}

In addition to the three main groups of studies, Thanert et al.\textsuperscript{48} was focused on children with surgically induced short bowel syndrome (SBS) making it distinct from other studies. Neither overall resistance gene load or alpha diversity of ARGs differed among children exposed or unexposed to antibiotics in the previous month in this study.

\textbf{Potential sources of bias in included studies}

Our bias assessment revealed some potential sources of bias from all included studies related to their assessment of antibiotic exposure and the resistome (Figure\textsuperscript{2} and Table S3). The predominant potential bias identified was bias due to the selective reporting of results, which was heavily affected by lack of publicly available protocols or statistical analysis plans for observational studies. While we amended the ROBINS-I guidelines for observational studies to account for particular challenges facing resistome studies including widespread unmeasured intra- and inter-individual variation (see Supplement), among observational studies, most studies presented potential for unmeasured confounding of the association between antibiotic exposures and the resistome. Likewise, while all studies measured the same ARGs across antibiotic exposed and unexposed children, some studies took samples at a different time,\textsuperscript{45,46,66} or more frequently,\textsuperscript{14,15,48,63} in antibiotic exposed children.

\textbf{Discussion}

While antibiotic exposures are known to have unintended side effects, limited prior research has assessed the impact of antibiotics on the gut resistome. As children are frequently exposed to antibiotics and childhood represents a sensitive window of microbiome development, it is necessary for researchers to study the effects of antibiotic exposure on the gut resistome to create better antibiotic stewardship.
The objectives of this systematic review were to highlight the known impact of antibiotics on the gut resistome of young children, compare heterogeneity across study findings, identify current gaps in the field, and reveal potential bias across studies.

We found evidence that antibiotics frequently but not always impact the gut resistome of young children. These results are likely affected by the differences in the half-lives, dose, duration, and mechanism of action of the antibiotic exposure(s). Studies that assessed the effect of antibiotics on the overall resistance gene load found either no association or a positive association. Results for richness or alpha diversity were inconsistent with studies identifying that antibiotics led to a decreased or increased alpha diversity of ARGs while other studies identified no statistically significant association with some variation by time points measured. These results are similar to a systematic review that found antibiotics impact microbial diversity inconsistently depending on the antibiotic exposure and population. While this systematic review did not prioritize abundance changes of individual or classes of ARGs due to heterogeneity in reference databases and metrics used to assess individual ARGs, future research is needed to establish how these overall resistome changes translate to specific antibiotic-resistant organisms.

In this systematic literature review, we identified three main groups of studies. These groups represent significantly different populations with distinct disease burdens and indications for antibiotic exposures. Results for our main outcomes differed by study group, but it’s worthwhile to mention that likely the strongest evidence that antibiotics impact the resistome come from the Group 1 studies due to the combination of randomization and direct observation of antibiotic exposures. Regardless, in crafting resistome-conscious antibiotic stewardship practices, a focused assessment of children from each population will likely offer the most opportunities for impact. This is an especially important consideration for studies that did not assess prophylactic antibiotic exposure that may be affected by confounding by indication (i.e., the effect of the antibiotic vs. the effect of the infection). Beyond these study groupings, there is a dearth of knowledge available to evaluate if antibiotics impact the gut resistome in young children. Additional studies assessing associations in populations from additional geographies and with varying diseases or disease risks (e.g., type I diabetes, irritable bowel disease, and cystic fibrosis) may help clarify the overall effects of antibiotics to the child gut resistome.

While this systematic review was able to expose the impact of antibiotics on the gut resistome of young children, assess potential biases in the field, and identify gaps in the literature, there were some limitations. One primary limitation we noted was the lack of consistency in reported information for both the exposure and outcomes of interest. This heterogeneity prevented formal meta-analysis of the data. Even without formal meta-analysis, we were able to identify consistent trends in the association for overall resistance gene load and different resistome alpha diversity trends by study grouping. This heterogeneity and lack of standardization in the microbiome and resistome field is an ongoing concern, but guidelines, such as the STORMS checklist could be beneficial. An additional limitation of this study was that we only used publicly available data. This decision was made as we felt that our goals were not oriented to quantify an exact metric for our outcomes. However, this did have an impact on our bias assessment. In particular, based on ROBINS-I criteria, many observational studies received a “Serious” categorization for potential bias due to selective reporting as many had no publicly available protocol. While there is certainly potential for much bias based on not having an advanced protocol, it’s worthwhile to emphasize that potential does not necessary equate to actualized bias. An individual-level meta-analysis of antibiotic and resistome data pulled from a database of studies that is able to standardize and normalize metrics would provide a better quantitative measurement with less potential bias.

Another limitation of this systematic review is that it did not focus on the abundance of individual ARGs nor ARG classes. As antibiotic exposure is likely to have heterogeneous effects on different ARGs, not all ARGs confer the same risk of leading to an antibiotic-resistant infection, and there are variable definitions of the number of ARGs that define the resistome, additional systematic reviews could help disentangle the impact of antibiotics on specific components of the resistome of young children. Lastly, we did not conduct a formal publication bias analysis per protocol, but there is evidence that this could be a concern. In particular, nine studies were excluded from our...
systematic review due to limited information tying the exposure and outcome of interest for this systematic review together.68,77–84 For these studies, we also could not identify publicly available individual-level data for re-analysis. Some studies likely did not focus on the association between antibiotic exposure and resistome outcomes due to a small sample size among antibiotic exposed children68,82,84 and thus would not have been powered to conduct a significant analysis. Without knowledge of protocols set in advance of data analysis, it’s unclear if other studies focused on antibiotic exposure due to a lack of association identified or because the authors prioritized assessing other exposure-outcome associations. Additional studies may clarify the associations discussed in this review, as we identified five clinical trials85–89 that are planning to incorporate information on the exposure and outcome of interest.

Conclusion

This systematic review found clear evidence that antibiotics impact the gut resistome of young children, but that additional studies are needed to evaluate the duration and extent. Potential bias across these studies is high with selective reporting of results and confounding major concerns that contributed to low confidence in the quality of quantitative evidence in this review. Additional studies in the field could help identify ideal antibiotic stewardship practices that consider the heterogeneity of the resistome in every population.

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Authors’ contributions

RML and AGH designed the research. RML, JCM, MRK, and AGH designed the study protocols. RML, DBK, JL, and HCW performed the data extraction and bias assessments. RML conducted any data re-analysis and wrote the initial manuscript draft. All authors reviewed the manuscript.

Disclosure statement

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Data availability statement

All data used in this manuscript is publicly available. The R Markdown script is available on GitHub (https://github.com/hoenlab/Antibiotics-on-Gut-Resistome-of-Young-Children-Systematic-Review.git). A template data extraction form is available in the Supplement and data extraction forms for each study are available upon request to the corresponding author.

References

1. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, Han C, Bisignano C, Rao P, Wool E, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–655. doi:10.1016/s0140-6736(21)02724-0.
2. Laxminarayan R, Matsoso P, Pant S, Brower C, Rottingen J-A, Klugman K, Davies S. Access to effective antimicrobials: a worldwide challenge. Lancet. 2016;387(10014):168–175. doi:10.1016/S0140-6736(15)00474-2.
3. Laxminarayan R, Van Boeckel T, Frost I, Kariuki S, Khan EA, Limmathurotsakul D, Larsson DGJ, Levy-Hara G, Mendelson M, Outterson K, et al. The lancet infectious diseases commission on antimicrobial resistance: 6 years later. Lancet Infect Dis. 2020;20(4):e51–e60. doi:10.1016/S1473-3099(20)30003-7.
4. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet. 2005;365(9459):579–587. doi:10.1016/S0140-6736(05)70799-6.
5. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309–318. doi:10.1179/2047773215Y.0000000030.
6. Mack I, Sharland M, Berkley JA, Klein N, Malhotra-Kumar S, Bielicki J. Antimicrobial resistance following azithromycin mass drug administration: potential surveillance strategies to assess public health impact. Clin Infect Dis. 2020;70(7):1501-1508. doi:10.1093/cid/ciz893.

7. Martínez JL, Coque TM, Baquero F. What is a resistance gene? Ranking risk in resistomes. Nat Rev Microbiol. 2015;13(2):116–123. doi:10.1038/nrmicro3399.

8. Stecher B, Maier L, Hardt WD. "Blooming" in the gut: how dysbiosis might contribute to pathogen evolution. Nat Rev Microbiol. 2013;11(4):277–284. doi:10.1038/nrmicro2989.

9. Bloom DE, Black S, Salisbury D, Rappuoli R. Antimicrobial resistance and the role of vaccines. Proc Natl Acad Sci. 2018;115(51):12868–12871. doi:10.1073/pnas.1717571115.

10. Spiro DM, Tay K-Y, Arnold DH, Dziura JD, Baker MD, Shapiro ED. Wait-and-see prescription for the treatment of acute otitis media: a randomized controlled trial. J Am Med Assoc. 2006;296(10):1235–1241. doi:10.1001/jama.2006.101235.

11. Feuerstadt P, Louie TJ, Lashner B, Wang EEL, Diao L, Bryant JA, Sims M, Kraft CS, Cohen SH, Berenson CS, et al. SER-109, an Oral Microbiome Therapy for Recurrent Clostridioides difficile Infection. N Engl J Med. 2022;386(3):220–229. doi:10.1056/nejmoa2106516.

12. Salyers AA, Gupta A, Wang Y. Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends Microbiol. 2004;12(9):412–416. doi:10.1016/j.tim.2004.07.004.

13. Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol. 2013;13(11):790–801. doi:10.1038/nri3535.

14. Gasparini AJ, Wang B, Sun X, Kennedy EA, Hernandez-Leyva A, Ndoo IM, Tarr PI, Warner BB, Dantas G. Persistent metagenomic signatures of early-life hospitalization and antibiotic treatment in the infant gut microbiota and resistome. Nat Microbiol. 2019;4(12):2285–2297. doi:10.1038/s41564-019-0550-2.

15. Yassour M, Vatanen T, Siljander H, Hämäläinen A, Hamalainen A, Al E, Franzosa EA, Vlamakis H, Huttenhower C, Gevers D. Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability. Sci Trans Med. 2016;8(343):1173–1178. doi:10.1126/scitranslmed.aad0917.

16. Schwartz DJ, Langdon AE, Dantas G. Understanding the impact of antibiotic perturbation on the human microbiome. Genome Med. 2020;12(82):1–12. doi:10.1186/s13073-020-00782-x.

17. Ho J, Kit Yeoh Y, Barua N, Chen Z, Lui G, Wong SH, Yang X, Chan MC, Chan PK, Hawkey PM, et al. Systematic review of human gut resistome studies revealed variable definitions and approaches. Gut Microbes. 2020;12(1):e1700755. doi:10.1080/19490976.2019.1700755.

18. Escher NA, Muhummed AM, Hattendorf J, Vonaesch P, Zinsstag J. Systematic review and meta-analysis of integrated studies on antimicrobial resistance genes in Africa—A One Health perspective. Trop Med Int Heal. 2021;26(10):1153–1163. doi:10.1111/tmi.13642.

19. Stam J, Van Stuijvenberg M, Grüber C, Mosca F, Arslanoglu S, Chirico G, Braegger CP, Riedler J, Boehm G, Sauer PJ, et al. Antibiotic use in infants in the first year of life in five European countries. Acta Paediatr Int J Paediatr. 2012;101(9):929–934. doi:10.1111/j.1651-2227.2012.02728.x.

20. Zhang T, Smith MA, Camp PG, Shajari S, MacLeod SM, Carleton BC. Prescription drug dispensing profiles for one million children: a population-based analysis. Eur J Clin Pharmacol. 2013;69(3):581–588. doi:10.1007/s00228-012-1343-1.

21. Klinow AC, Stürmer T, Lund JL, Pedersen L, Kappelman MD, Daniels JL, Frosliev T, Mack CD, Serensen HT. Trends in antibiotic use by birth season and birth year. Pediatrics. 2017;140(3):e20170441. doi:10.1542/peds.2017-0441.

22. Dawson-Hahn EE, Rhee KE. The association between antibiotics in the first year of life and child growth trajectory. BMC Pediatr. 2019;19(1):1–9. doi:10.1186/s12887-018-1363-9.

23. Fleming-duke RA, Demirjian A, Bartoces M, Roberts RM Jr, Hicks LA, Hicks LA. Variations in antibiotic and azithromycin prescribing by geography and specialty – United States, 2013. Pediatr Infect Dis J. 2019;37(1):52–58. doi:10.1097/INF.0000000000001708. Variations.

24. Jackson C, Hsia Y, Bielicki JA, Ellis S, Stephens P, Wong ICK, Sharland M. Estimating global trends in total and childhood antibiotic consumption, 2011-2015. BMJ Glob Heal. 2019;4(1):1–9. doi:10.1136/bmjgh-2018-001241.

25. Boolchandani M, D’Souza AW, Gautam D. Sequencing-based methods and resources to study antimicrobial resistance. Nat Rev Genet. 2019;20(6):356–370. doi:10.1038/s41576-019-0108-4.

26. Cabral DJ, Penumutchu S, Reinhart EM, Zhang C, Korry BJ, Wurster JI, Nilson R, Guang A, Sano WH, Rowan-Nash AD, et al. Microbial Metabolism Modulates Antibiotic Susceptibility within the Murine Gut Microbiome. Cell Metab. 2019;30(4):800–823.e7. doi:10.1016/j.cmet.2019.08.020.

27. Korpela K, Salonen A, Saxen H, Nikkonen A, Peltola V, Jaakkola T, de Vos W, Kolho K-L. Antibiotics in early life associate with specific gut microbiota signatures in a prospective longitudinal infant cohort. Pediatr Res. 2020;88(3):438–443. doi:10.1038/s41390-020-0761-5.

28. McDonnell L, Gilkes A, Ashworth M, Rowland V, Harries TH, Armstrong D, White P. Association between antibiotics and gut microbiome dysbiosis in children: systematic review and meta-analysis. Gut
reproducible in assessing the quality of evidence of quantitative evidence syntheses. J Clin Epidemiol. 2013;66(7):736–742.e5. doi:10.1016/j.cej.2013.02.004.

51. Schünemann H, Brozek J, Guyatt G, Oxman A. GRADE Working Group. October 2013. Handbook for Grading the Quality of Evidence and the Strength of Recommendations Using the GRADE Approach. https://gdt.graedeuro.org/app/handbook/handbook.html.

52. Oldenburg CE, Hintewirth A, Sié A, Coulibaly B, Ouermi L, Dah C, Tapsoba C, Cummings SL, Zhong L, Chen C, et al. Gut resistome after oral antibiotics in preschool children in Burkina Faso: a randomized, controlled trial. Clin Infect Dis. 2020;70(3):525–527. doi:10.1093/cid/ciz455.

53. Oldenburg CE, Hintewirth A, Sié A, Coulibaly B, Ouermi L, Dah C, Tapsoba C, Cummings S, Zhong L, Chen C, Sarkar S, Barnighaussen T, Liemtn T, Keenan J, Doan T. 2019. Gut resistome after oral antibiotics in preschool children in Burkina Faso: A randomized controlled trial. ASTMH:Maryland, USA:101 457. doi:10.1111/j.1752-7325.1950.tb01243.x.

54. US NLM. 2017. Antibiotic resistance and microbiome in children aged 6-59 months in Nouna, Burkina Faso (A R M C A). https://clinicaltrials.gov/show/NCT03187834.

55. Bourke CD, Evans C. Cotrimoxazole Prophylaxis Selects for Antimicrobial Resistance in Human Immunodeficiency Virus–Exposed, Uninfected Infants. Clin Infect Dis an off Publ Infect Dis Soc Am. 2020;71(11):2869–2871. doi:10.1093/cid/ciz1193.

56. Daniels B, Coutoudis A, Moody-Govender E, Mulol H, Spooner E, Kiepiela P, Reddy S, Zako L, Ho NT, Kuhn L, et al. Effect of co-trimoxazole prophylaxis on morbidity and mortality of HIV-exposed, HIV-uninfected infants in South Africa: a randomised controlled, non-inferiority trial. Lancet Glob Heal. 2019;7(12):e1717–e1727. doi:10.1016/S2214-109X(19)30422-X.

57. Doan T, Arzika AM, Hintewirth A, Maliki R, Zhong L, Cummings S, Sarkar S, Chen C, Porco TC, Keenan JD, et al. Macrolide Resistance in MORDOR I — a Cluster-Randomized Trial in Niger. N Engl J Med. 2019;380(23):2271–2273. doi:10.1056/nejmc1901535.

58. Doan T, Hintewirth A, Worden L, Arzika AM, Maliki R, Abdou A, Kane S, Zhong L, Cummings SL, Sakar S, et al. Gut microbiome alteration in MORDOR I: a community-randomized trial of mass azithromycin distribution. Nat Med. 2019;25(9):1370–1376. doi:10.1038/s41591-019-0533-0.

59. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 2015;17(5):690–703. doi:10.1016/j.chom.2015.04.004.

60. Hourigan SK, Subramanian P, Hasan NA, Ta A, Klein E, Chettout N, Huddleston K, Deopujari V, Levy S, Baveja R, et al. Comparison of infant gut and skin microbiota, resistome and virulome between neonatal intensive care unit (NICU) environments. Front Microbiol. 2018;9. doi:10.3389/fmicb.2018.01361.

61. Moore AM, Ahmadi S, Patel S. Gut resistome development and in healthy twin pairs in the first year of life. Microbiome. 2015;3(27):1–10. doi:10.1186/s40168-015-0090-9.

62. Gasparrini AJ, Crofts TS, Gibson MK, Tarr PI, Warner BB, Dantas G. Antibiotic perturbation of the preterm infant gut microbiome and resistome. Gut Microbes. 2016;7(5):443–449. doi:10.1080/19490976.2016.1218584.

63. Gibson MK, Wang B, Ahmadi S, Burnham CD, Tarr PI, Warner BB, Dantas G. Developmental dynamics of the preterm infant gut microbiota and antibiotic resistome. Nat Microbiol. 2016;301903:1–25. doi:10.1038/nmicrobiol.2016.24.

64. Reyman M, van Houten MA, Watson RL, Chu MLJN, Arp K, de Waal WJ, Schiering I, Plötz FB, Willems RJL, van Schaik W, et al. Effects of early-life antibiotics on the developing infant gut microbiome and resistome: a randomized trial. Nat Commun. 2022;13(1):893. doi:10.1038/s41467-022-28525-z.

65. Netherlands Trial Register Zebra Study. https://trialsearch.who.int/TrialID=NTR5119. 2015.

66. Rose G, Shaw AG, Sim K, Wooldridge DJ, Li M-S, Gharbia S, Misra R, Kroll JS. Antibiotic resistance potential of the healthy preterm infant gut microbiome. PeerJ. 2017;5:e2928. doi:10.7717/peerj.2928.

67. Mustonen N, Silijander H, Peet A, Tillmann V, Härkönen T, Ilenen J, Hyötyn H, Knip M, Koski K, Koski M, et al. Early childhood infections and the use of antibiotics and antipyretic-analgesics in Finland, Estonia and Russian Karelia. Acta Paediatr Int J Paediatr. 2019;108(11):2075–2082. doi:10.1111/apa.14874.

68. Lebeaux RM, Coker MO, Dade EF, Palys TJ, Morrison HG, Ross BD, Baker ER, Karagas MR, Madan JC, Hoen AG, et al. The infant gut resistome is associated with E. coli and early-life exposures. BMC Microbiol. 2021;21(201):1–18. doi:10.1186/s12866-021-02129-x.

69. Pärnänen K, Karkman A, Hultman J, Lyra C, Bengtsson-Palme J, Larsson DGJ, Rautava S, Isolauri E, Salminen S, Kumar H, et al. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat Commun. 2018;9(3891):1–11. doi:10.1038/s41467-018-06393-w.

70. Casaburi G, Duar RM, Vance DP, Mitchell R, Contreras L, Frese SA, Smilowitz JT, Underwood MA. Early-life gut microbiome modulation reduces the
abundance of antibiotic-resistant bacteria. Antimicrob Resist Infect Control. 2019;8(131):1–18. doi:10.1186/s13756-019-0583-6.

71. Yang L, Bajinka O, Jarju PO, Tan Y, Taal AM, Ozdemir G. The varying effects of antibiotics on gut microbiota. AMB Express. 2021;11(1). doi:10.1186/s13668-021-01274-w.

72. Joseph KS, Mehrabadi A, Lisonkova S. Confounding by Indication and Related Concepts. Curr Epidemiol Reports. 2014;1(1):1–8. doi:10.1007/s40471-013-0004-y.

73. Letouzey M, Foix-L’Hélias L, Boileau P, Lorthe E. Association of early antibiotic exposure and necrotizing enterocolitis: causality or confounding bias? J Pediatr. 2020;226(November):315–316. doi:10.1016/j.jpeds.2020.07.060.

74. Nearing JT, Comeau AM, Langille MGI. Identifying biases and their potential solutions in human microbiome studies. Microbiome. 2021;9(1):1–22. doi:10.1186/s40168-021-01059-0.

75. Mirzayi C, Renson A, Furlanello C, Sansone S-A, Zohra F, Elsafoury S, Geistlinger J, Kasselman J, Eckenrode K, van de Wijgert J, et al. Reporting guidelines for human microbiome research: the STORMS checklist. Nat Med. 2021;27(11):1885–1892. doi:10.1038/s41591-021-01552-x.

76. Chong J, Liu P, Zhou G, Xia J. Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat Protoc. 2020;15(3):799–821. doi:10.1038/s41596-019-0264-1.

77. Taft DH, Liu J, Maldonado-Gomez MX, Akre S, Huda MN, Ahmad SM, Stephensen CB, Mills DA. Bifidobacterial Dominance of the Gut in Early Life and Acquisition of Antimicrobial Resistance. mSphere. 2018;3(5). doi:10.1128/msphere.00441-18.

78. Loo EXL, Zain A, Yap GC, Purbojati RW, Drautz-Moses DI, Koh YQ, Chong YS, Tan KH, Gluckman PD, Yap F, et al. Longitudinal assessment of antibiotic resistance gene profiles in gut microorganisms of infants at risk of eczema. BMC Infect Dis. 2020;20(1):1–12. doi:10.1186/s12879-020-05000-y.

79. Ribado JV, Ley C, Hagerty TD, Tkachenko E, Bhatt AS, Parsonnet J. Household triclosan and triclocarban effects on the infant and maternal microbiome. EMBO Mol Med. 2017;9(12):1732–1741. doi:10.15252/emmm.201707882.

80. Nguyen M, Holdbrooks H, Mishra P, Abrantes MA, Eskew S, Garma M, Oca C, McGluckin C, Hein CB, Mitchell RD, et al. Impact of Probiotic B. infantis EVC001 Feeding in Premature Infants on the Gut Microbiome, Nosocomially Acquired Antibiotic Resistance, and Enteric Inflammation. Front Pediatr. 2021;9. doi:10.3389/fped.2021.618009.

81. Shao Y, Forster SC, Tsalki E, Vervier K, Strang A, Simpson N, Kumar N, Stares MD, Rodger A, Brocklehurst P, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. Nature. 2019;574(7776):117–121. doi:10.1038/s41586-019-1560-1.

82. Sosa-Moreno A, Comstock SS, Sugino KY, Ma TF, Paneth N, Davis Y, Olivero R, Schein R, Maurer J, Zhang L, et al. Perinatal risk factors for fecal antibiotic resistance gene patterns in pregnant women and their infants. PLoS One. 2020;15(6):1–21. doi:10.1371/journal.pone.0234751.

83. Yassour M, Jason E, Hogstrom LJ, Arthur TD, Tripathi S, Siljander H, Selvenius J, Okarinen S, Hyötý H, Virtanen SM, et al. Strain-Level Analysis of Mother-to-Child Bacterial Transmission during the First Few Months of Life Short Article Strain-Level Analysis of Mother-to-Child Bacterial Transmission during the First Few Months of Life. Cell Host Microbe. 2018;24(1):146–154. doi:10.1016/j.chom.2018.06.007.

84. Leggett RM, Alcon-Giner C, Heavens D, Cairn S, Brook TC, Kujawska M, Martin S, Peel N, Axford-Palmer H, Hoyles L, et al. Rapid MinION profiling of preterm microbiota and antimicrobial-resistant pathogens. Nat Microbiol. 2020;5(3):430–442. doi:10.1038/s41564-019-0626-z.

85. US NLM. 2020. Effects of mass drug administration of azithromycin on mortality and other outcomes among 1–11 month old infants in Mali (LAKANA) https://clinicaltrials.gov/ct2/show/NCT04424511.

86. US NLM. MORDOR II Burkina Faso: longitudinal trial (GAMIN) https://clinicaltrials.gov/ct2/show/NCT03676751. 2018.

87. US NLM. 2020. Single dose azithromycin to prevent cholera in children https://clinicaltrials.gov/ct2/show/NCT04326478.

88. Platform ICTR. Antibiotic prophylaxis for children with recurrent respiratory infections: towards evidence-based guidelines - AppRoACH study. 2018.

89. Platform ICTR. Pilot study on the impact of timing of antimicrobial prophylaxis for elective Caesarean section at term on infant gut microbiome https://trialssearch.who.int/Trial2.aspx?TrialID=DRKS00025305. 2019.