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MAJOR ARTICLE

Each Additional Day of Antibiotics Is Associated With Lower Gut Anaerobes in Neonatal Intensive Care Unit Patients

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Background. Discontinuation of inappropriate antimicrobial therapy is an important target for stewardship intervention. The drug and duration-dependent effects of antibiotics on the developing neonatal gut microbiota needs to be precisely quantified.

Methods. In this retrospective, cross-sectional study, we performed 16S rRNA sequencing on stool swab samples collected from neonatal intensive care unit patients within 7 days of discontinuation of therapy who received ampicillin and tobramycin (AT), ampicillin and cefotaxime (AC), or ampicillin, tobramycin, and metronidazole (ATM). We compared taxonomic composition within term and preterm infant groups between treatment regimens. We calculated adjusted effect estimates for antibiotic type and duration of therapy on the richness of obligate anaerobes and known butyrate-producers in all infants.

Results. A total of 72 infants were included in the study. Term infants received AT (20/28; 71%) or AC (8/28; 29%) with median durations of 3 and 3.5 days, respectively. Preterm infants received AT (32/44; 73%) or ATM (12/44; 27%) with median durations of 4 and 7 days, respectively. Compositional analyses of 67 stool swab samples demonstrated low diversity and dominance by potential pathogens. Within 1 week of discontinuation of therapy, each additional day of antibiotics was associated with lower richness of obligate anaerobes (adjusted risk ratio [aRR], 0.84; 95% confidence interval [CI], .73–.95) and butyrate-producers (aRR, 0.82; 95% CI, .67–.97).

Conclusions. Each additional day of antibiotics was associated with lower richness of anaerobes and butyrate-producers within 1 week after therapy. A longitudinally sampled cohort with preexposure sampling is needed to validate our results.

Keywords. gut microbiome; neonates; anaerobes; antibiotic duration; stewardship.

Continuation of antibiotic therapy in the absence of infection is a common form of inappropriate antibiotic prescription in the neonatal intensive care unit (NICU) [1, 2]. Prolonged antimicrobial exposure (greater than 3 or 5 days) is associated with short-term complications including necrotizing enterocolitis (NEC), hospital-acquired infection, and mortality [3, 4], as well as long-term health outcomes including obesity, allergy, and inflammatory bowel disease [5–7]. Discontinuation of unnecessary antibiotics is an important target for stewardship, yet some physicians perceive that discontinuing empiric antibiotic therapy is difficult, even in the absence of infection [8, 9]. This is highlighted by the significant variation in antibiotic prescribing practices across NICUs [10].

Infancy represents a critical time for gut microbiota development in which obligate anaerobes and butyrate-producers are acquired [11]. Anaerobes influence the education and maturation of the host immune system [12], provide colonization resistance against pathogens and antimicrobial-resistant organisms [13], and play an important role in metabolism, including the production of short-chain fatty acids such as butyrate [14]. Microbially produced butyrate promotes gut epithelial barrier function, physiologic mucosal hypoxia, proliferation of health-associated anaerobes, and suppression of facultative anaerobes such as *Enterobacteriaceae* [15, 16], as well as local immune system homeostasis [17].

Research investigating the duration-specific impact of commonly used antimicrobial regimens on the developing infant gut microbiota is limited and may inform therapeutic decision-making in the NICU. In this study, we compared the effects of ampicillin and tobramycin (AT), ampicillin and cefotaxime

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(AC), and ampicillin, tobramycin, and metronidazole (ATM) and the duration of treatment on end-of-therapy gut microbial community diversity and strict anaerobe composition in NICU infants.

METHODS

Study Design and Participants

We conducted a retrospective, cross-sectional study of infants who had participated in a prospective cohort evaluating *Clostridioides difficile* colonization in the NICU (unpublished work). In this cross-sectional study, we used stool swab samples taken within 1 week (1–7 days) of antibiotic exposure to compare gut microbiota diversity, anaerobe composition, and, as a secondary outcome, butyrate-producer composition in NICU infants. Infants were included in the study if they received at least 1 dose of AT, AC, or ATM. Infants were excluded if they did not have a stool swab sample taken within 1–7 days after the discontinuation of therapy or had previous documented antibiotic prescription prior to the start of the initial antibiotic prescription. The Hospital for Sick Children Research Ethics Board approved the study.

Clinical Information Collection

Patients who received antibiotics were assessed prospectively as part of twice weekly antimicrobial stewardship rounds by the clinical and antimicrobial stewardship teams. Information on antibiotic indication was collected and documented prospectively. NICU clinicians prescribed antibiotics in accordance with local guidelines, irrespective of the study. Based on these guidelines, AT is an empiric regimen for sepsis in newborns, AC is an empiric regimen for central nervous system infection or in cases where tobramycin is contraindicated (ie, renal dysfunction), and ATM is used for confirmed or suspected gastrointestinal (GI) infections, including NEC and other nonnecrotizing GI pathology. The indications for antibiotics were classified based on the presumed site of infection, if known. In patients without a specific source, sepsis was selected. GI/abdominal source indications included NEC and other nonnecrotizing GI infections or when antibiotics were used for GI tract surgical prophylaxis (eg, anorectal malformations, bowel obstruction). For this study, antibiotic dispensing data were used and obtained from the pharmacy system. The number of antibiotic days was defined as the number of calendar days on which the patient received at least 1 dose of antibiotic.

Sample Collection and Processing

From 1 November 2014 to 30 April 2015, stool swabs were collected from the diapers of NICU patients using FLOQSwabs (Copan, Brescia, Italy) and subsequently stored in eNAT (Copan, Brescia, Italy) nucleic acid preservation buffer at –80°C. Stool samples (200 µL) were subject to DNA extraction using the DNeasy PowerSoil Kit (cat. 12888-100, Qiagen, Carlsbad, CA) and stored at –80°C.

16s rRNA Sequencing and Data Processing

DNA samples were sequenced at the Centre for the Analysis of Genome Evolution and Function at the University of Toronto. The V4 hypervariable region of the 16S rRNA gene was amplified using a universal forward primer and a uniquely bar-coded reverse primer, as described previously [18]. The pooled sample library was loaded on to the Illumina MiSeq (Illumina, San Diego, CA) for paired-end 150 base pair sequencing using V2 chemistry.

Raw sequences were processed using Qiime2 v.2018.4 [19]. Demultiplexed paired-end sequences were subject to DADA2 for quality filtering, denoising, and chimera removal [20]. Taxonomy was assigned to amplicon sequence variants (ASVs) using a naive Bayes classifier trained on the GreenGenes 13_8 99% operational taxonomic units [21]. ASVs that were classified at the kingdom level only (k__Bacteria;_) were filtered from each sample. Samples were rarified to 3736 sequences per sample, as this sequencing depth retained the most samples and discarded those under 1000 sequences. Sequences were deposited in the SRA database (accession: PRJNA532426).

16s rRNA Gene Quantitative Polymerase Chain Reaction for Relative Bacterial Density

Bacterial density of each fecal sample and DNA extraction negative controls were measured using quantitative polymerase chain reaction, with the forward primer (5'-TCCTACGGGAGGCAGCAGT-3'), the reverse primer (5'-GGACTACCAGGGTATCTAATCCTGTT-3'), and probe (FAM-5'-CGTATTACCGCGGCTGCTGGCAC-3'-NFQ-MGB) (Applied Biosystems). The 16S rRNA gene density relative to the lowest density sample was calculated using the $2^{-(\text{delta CT})}$ calculation, then log-transformed for each sample. We applied a bacterial density cutoff at a cycle threshold (CT) value of 40. Any samples ≥40 CT were considered to have no detectable bacteria.

Microbiota Analysis

Overall diversity in each sample was summarized by phylum, genus-level obligate anaerobes, family-level butyrate-producers, and per sample dominant taxa. We defined dominance as greater than 30% relative abundance. The Shannon diversity index, a measure of both richness (number of unique taxa) and taxonomic evenness, as well as the Chao1 index, an estimated measure of taxonomic richness, were calculated for each sample using a feature table collapsed to level 6 (generally the genus). These analyses were conducted in Qiime2 v.2018.4 [19].

Anaerobe Classification and Quantification

A feature table collapsed to level 6 was used to identify obligate anaerobes. First, the maximum relative abundance for each

taxon was identified across samples. Taxa that did not contribute 0.1% relative abundance in at least 1 sample were excluded. We used *Bergey's Manual of Systematic Bacteriology*, volumes 2–5, to manually classify taxa as obligate anaerobes based on descriptors in the manuals, such as "strictly anaerobic," "anaerobic," or "obligate anaerobe" [22–26]. Facultative anaerobes, aerobes, chloroplast, and proteobacteria classified as mitochondria at the genus level were excluded. ASVs that were annotated to a taxonomic level that did not allow for specific identification of obligate anaerobes were subject to the basic local alignment search tool on the National Centre for Biotechnology Information website to be further classified as "probable aerobe," "probable anaerobe," or "unknown." Supplementary Table 1 provides information on the classification of taxa identified. As a secondary outcome, observed butyrate-producers were quantified at the family level based on a list of known butyrate-producers, including *Bifidobacteriaceae*, *Bacteroidaceae*, *Porphyromonadaceae*, *Eubacteriaceae*, *Lachnospiraceae*, *Ruminococcaceae*, *Erysipelotrichaceae*, and *Fusobacteriaceae* [27]. The relative abundance and richness of obligate anaerobes and butyrate-producers in each sample were quantified.

Statistical Analyses

Samples were grouped by gestational age, where preterm infants were defined as less than 37 weeks gestation, then stratified by treatment regimen received. Additional subgroup analyses based on delivery mode, exposure to mother's breast milk, and indication were performed. We log-transformed the relative abundances of taxa to compare between treatment groups. To do this, a single sequence read was added to each taxon (1 sequence represents the lower limit of sequencing detection) to account for taxa with zero sequences; the relative abundances were recalculated and then log-transformed. The Shannon diversity index, Chao1 index, relative abundances, and richness of obligate anaerobes were compared between treatment groups using the nonparametric Mann-Whitney *U* test. The Fisher exact test was used to compare proportions of dominant taxa between treatment groups. Additionally, we used simple linear regression to assess the relationship between previous antibiotic duration and the richness of obligate anaerobes and butyrate-producers. The Mann-Whitney *U* test, Fisher exact test, and simple linear regression were performed in GraphPad Prism v.7.0.3.

Multivariate Regression Analysis

The effects of antibiotic treatment type (AC or ATM vs AT) and antibiotic duration, measured as a continuous variable, on the richness of obligate anaerobes (primary outcome) and observed butyrate-producers (secondary outcome) in infant stool samples were adjusted for by using negative binomial regression (MASS package) and Poisson regression, respectively, in R version 3.5.1. We decided a priori to adjust for the following variables because of their known association with differences in neonatal gut microbiota diversity: gestational age (weeks) [28, 29], age at sampling (days) and time since antibiotic cessation (days) [11, 30], delivery mode (caesarean section or vaginal) [11, 31], indication (sepsis or GI/abdominal, including NEC, vs all other indications) [32, 33], and exposure to mother's breast milk [11, 30, 31] (at least 1 exposure prior to stool sample collection, excluding the collection day). Adjusted risk ratios (aRRs) were calculated for antibiotic type and antibiotic duration by exponentiating regression coefficients and associated 95% confidence intervals (CIs).

RESULTS

NICU Patient Characteristics

A total of 72 NICU infants were included (Table 1 outlines the patient characteristics). Term infants (n = 28) were born at a median gestational age of 39 weeks (range, 37–41 weeks) and preterm infants (n = 44) were born at a median gestational age of 32.5 weeks (range, 24–36 weeks). Term infants received either AT (20/28; 71%) or AC (8/28; 29%), whereas preterm infants received either AT (32/44; 73%) or ATM (12/44; 27%). The median duration of AT therapy was 3 (range, 1–8 days) and 4 days (range, 2–8 days) for term and preterm infants, respectively. The median duration of therapy for term infants who received AC therapy was 3.5 days (range, 2–8 days), whereas the median duration of ATM therapy in preterm infants was 7 days (range, 1–10 days). Of the infants who received ATM therapy, 100% (12/12) had suspected GI/abdominal indications, of which 42% (5/12) had suspected NEC. Five patients were removed from all microbiota analyses as 4 patients had fewer than 1000 sequences per sample and 1 term infant was 213 days old at admission (221 days at sampling) and likely not representative of the rest of the cohort, thus 67 of 72 infant samples were analyzed.

NICU Gut Microbiota Composition

Bacterial density varied by 8 logs between the highest and lowest density sample (Figure 1*D* and 1*H*). A total of 6 patients, 5 preterm infants in the AT group and 1 term infant in the AT group, had no detectable bacteria in their samples. The relative abundances of the phyla Proteobacteria and Firmicutes were high across all infant samples compared to the phyla Bacteroidetes and Actinobacteria (Supplementary Figure 1A). For samples with detectable bacteria, a large proportion (56/61; 92%) were dominated by at least 1 organism, including members of *Enterobacteriaceae*, *Staphylococcus* spp., or *Enterococcus* spp. (Figure 1*B* and 1*F*). We did not observe statistically significant differences in the presence of dominant taxa between treatment groups. The Shannon diversity index was low across all samples and similar in term and preterm infants between treatment

Table 1. Neonatal Intensive Care Unit Patient Characteristics

	Term Infants (n = 28)		Preterm Infants (n = 44)	
Characteristic	AT (n = 20)	AC (n = 8)	AT (n = 32)	ATM (n = 12)
Male sex, n (%)	9 (45)	5 (62)	16 (50)	7 (58)
Mean weight (standard deviation), g	3275 (±658)	3301 (±403)	1839 (±707)	1694 (±639)
Gestational age,[a] weeks	39 (37–41)	39 (37–40)	32 (25–36)	33 (24–36)
Age at sampling,[a] days	7 (3–29)	14.5 (4–28)	6.5 (3–221)	15 (5–35)
Caesarean delivery, n (%)	8 (40)	4 (57)[b]	18 (60)[c]	5 (42)
MBM, n (%)	15 (75)	5 (62)	23 (72)	9 (75)
Non-MBM,[d] n (%)	12 (60)	2 (25)	16 (50)	1 (8)
Total parenteral nutrition, n (%)	15 (75)	5 (62)	26 (81)	11 (92)
Antibiotic duration,[a] days	3 (1–8)	3.5 (2–8)	4 (2–8)	7 (1–10)
Sepsis, n (%)	6 (30)	2 (25)	18 (56)	0 (0)
Gastrointestinal/Abdominal,[e] n (%)	2 (10)	0 (0)	4 (12)	7 (58)
Necrotizing enterocolitis,[f] n (%)	0 (0)	0 (0)	0 (0)	5 (42)
Surgical prophylaxis, n (%)	2 (10)	0 (0)	2 (6)	0 (0)
Respiratory, n (%)	5 (25)	1 (12)	8 (25)	0 (0)
Central nervous system, n (%)	5 (25)	5 (62)	0 (0)	0 (0)

Abbreviations: AC, ampicillin and cefotaxime; AT, ampicillin and tobramycin; ATM, ampicillin; tobramycin, and metronidazole; MBM, mother's breast milk.

[a]Median (range).

[b]One missing value for caesarean delivery.

[c]Two missing values for caesarean delivery.

[d]Non-MBM includes formula, donor milk, and human milk fortifier.

[e]Includes only nonnecrotizing enterocolitis indications.

[f]Classified as a gastrointestinal/abdominal indication.

regimens received, as well between delivery modes, mother's breast milk exposure (yes/no), and indication (Supplementary Figures 1C and Figure 3A, D, and G). There was also no difference in the Chao1 index in term and preterm infants between treatment regimens received (Supplementary Figure 1D).

Obligate Anaerobe and Butyrate-producer Composition

Obligate anaerobe and butyrate-producer composition is summarized in term (Figure 1*C* and 1*D*) and preterm (Figure 1*G* and 1*H*) infant samples. A total of 20/67 (30%) infant samples had no detectable strict anaerobes, while in the samples that had at least 1 strict anaerobe, *Bacteroides* spp. members of the *Clostridiaceae* family, including *Clostridium* spp., as well as *Veillonella* spp. were common. As expected, anaerobe richness and relative abundance were lower in this antibiotic-treated term and preterm cohort compared to published data of healthy, term infants [11] (Supplementary Figure 2A–D). Obligate anaerobe and butyrate-producer richness was significantly higher in vaginally delivered infants compared to infants delivered by caesarean section ($P < .05$, Mann-Whitney) (Supplementary Figure 3B and C). The median relative abundance (Supplementary Figure 1B) and richness of anaerobes (Figure 2*A*) and butyrate-producers (Figure 3*A*) were similar in term and preterm infants between treatment regimens received. However, we observed an inverse relationship between antibiotic duration and the richness of obligate anaerobes (Figure 2*B*) and butyrate-producers (Figure 3*B*) 1 week postantimicrobial exposure across all infant samples as well as in term and preterm infants by treatment regimen received (Figure 2*C* and 2*D* and (Figure 3*C* and 3*D*).

Effect Estimates for Antibiotic Type and Duration on Anaerobe and Butyrate-producer Richness

Crude unadjusted and adjusted effect estimates are presented in Table 2; all adjusted variables are provided in Supplementary Table 2. After adjusting for delivery mode, indication, exposure to mother's breast milk, gestation age, age at time of sampling, and time since antibiotic cessation, we found that each additional day of antimicrobial therapy was associated with 16% lower (aRR, 0.84; 95% CI, .73–.95) richness of obligate anaerobes. Additionally, AC therapy was associated with 111% higher (aRR, 2.11; 95% CI, 1.07–4.10) richness of obligate anaerobes compared to AT therapy. However, there was no difference between AC and AT therapy in the crude unadjusted analysis (Table 2). We did not find a difference in the richness of obligate anaerobes after exposure to ATM therapy (aRR, 0.81, 95% CI. .27–2.62) compared to AT therapy (Table 2).

We also found that each additional day of antimicrobial therapy was associated with 18% lower (aRR, 0.82; 95% CI, .67–.97) richness of butyrate-producing families. However, we did not observe differences between richness of butyrate-producers and the antibiotic types ATM (aRR, 1.23; 95% CI, .29–6.56) or AC (aRR, 1.86; 95% CI, .76–4.24) compared to AT therapy (Table 2).

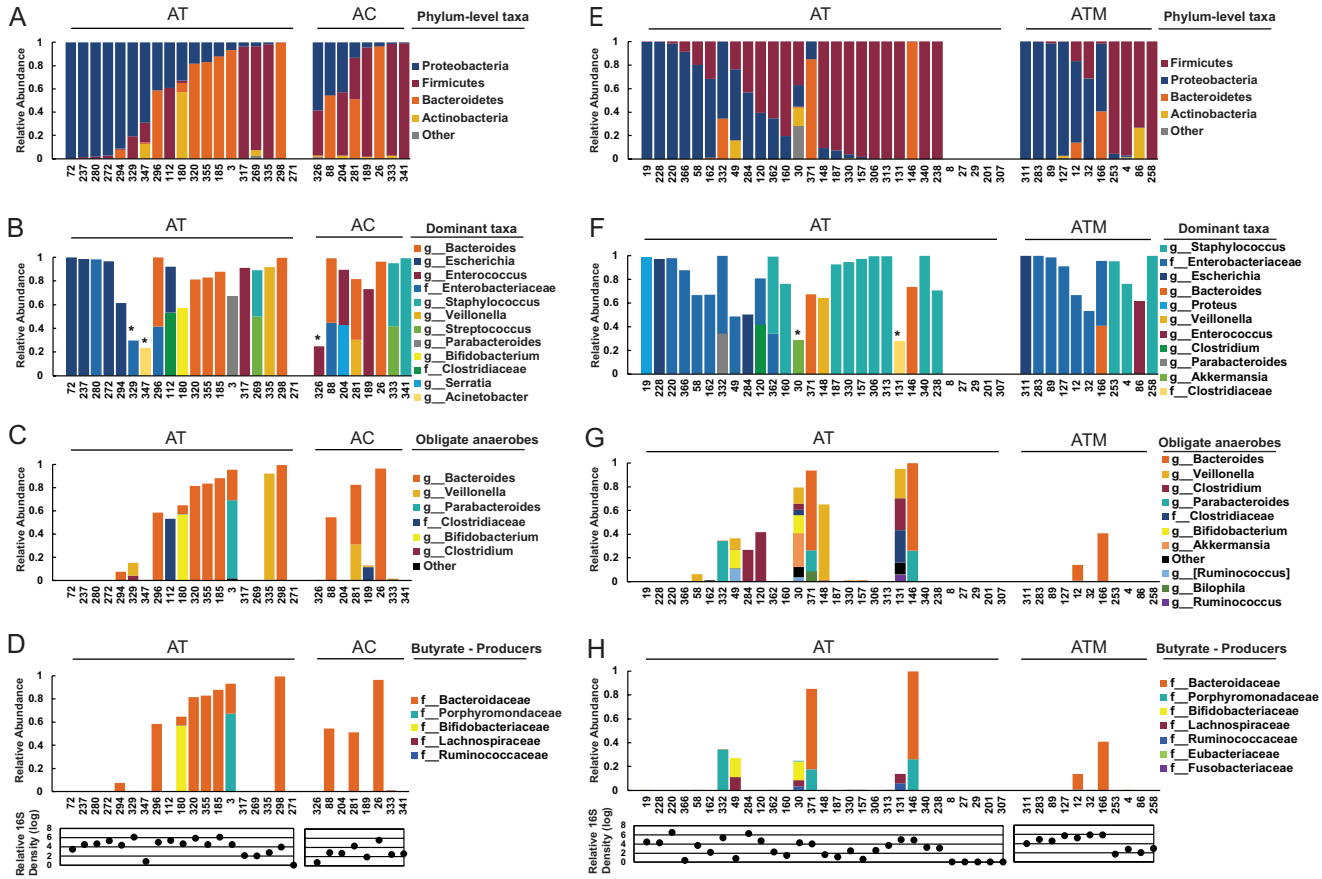

Figure 1. Compositional summary of stool swab samples in term (*A–D*) and preterm (*E–H*) neonatal intensive care unit patients 1 week postantimicrobial exposure, stratified by treatment regimen received: AT, AC, or ATM. *A* and *E*, The relative abundances of phyla that represented >1% of each infant sample. Phyla representing <1% were aggregated as "Other." *B* and *F*, The relative abundances of dominant genus-level taxa in each infant stool sample. We defined dominance as >30% relative abundance (* denotes the most abundant nondominant taxon <30%). *C* and *G*, The relative abundances of obligate anaerobes identified using *Bergey's Manual of Systematic Bacteriology* and the basic local alignment search tool. Obligate anaerobes representing <1% relative abundance were aggregated as "Other." *D* and *H*, The relative abundances of butyrate-producers identified at the family level. The log scale 16S rRNA gene density relative to the lowest density sample is plotted beneath each patient's sample. Abbreviations: AC, ampicillin and cefotaxime; AT, ampicillin and tobramycin; ATM, ampicillin, tobramycin, and metronidazole.

DISCUSSION

In this retrospective, observational cohort study of 67 evaluable NICU patients, each additional day of antibiotics was associated with 16% and 18% lower obligate anaerobe and butyrate-producers at the end of therapy, respectively. We expected that longer durations of therapy would be associated with a lower abundance of anaerobes in NICU patients within 1 week of therapy. A recent systematic review reported that studies that assessed the effects of duration on neonatal gut microbiota found that longer durations of therapy are associated with lower microbial diversity [34]. However, these studies focused on preterm infants alone and grouped duration of antibiotic treatment categorically. The studies that did assess anaerobe abundance postantimicrobial exposure focused on specific anaerobes such as *Clostridia* spp. and *Bifidobacterium* spp. [34]. A strength of our study is that we quantified anaerobic composition across gestational ages while estimating the effect of duration as a continuous variable on the richness of all identified anaerobes.

We hypothesized that the addition of metronidazole and the use of cefotaxime compared to tobramycin would result in reduced microbial diversity. We did not observe differences in alpha diversity, dominant taxon, or anaerobe/butyrate-producer relative abundance at the end of therapy between AT- and ATM-treated or AC-treated neonates. After adjusting for confounders, AC therapy was associated with greater richness of anaerobes compared to AT therapy. However, the number of evaluable infants who received ATM (n = 11) or AC (n = 7) in our study was small, and the effect of metronidazole or cefotaxime compared to tobramycin may be dependent on the initial composition of the gut microbiota, which we were unable to control for [35]. Importantly, antimicrobial susceptibility profiles among anaerobic strains and species may differ between individuals, which could potentially contribute to the heterogeneity of antibiotic effects [36].

There are several limitations to this study. First, study inclusion was based on antibiotic dispensing data, which may not

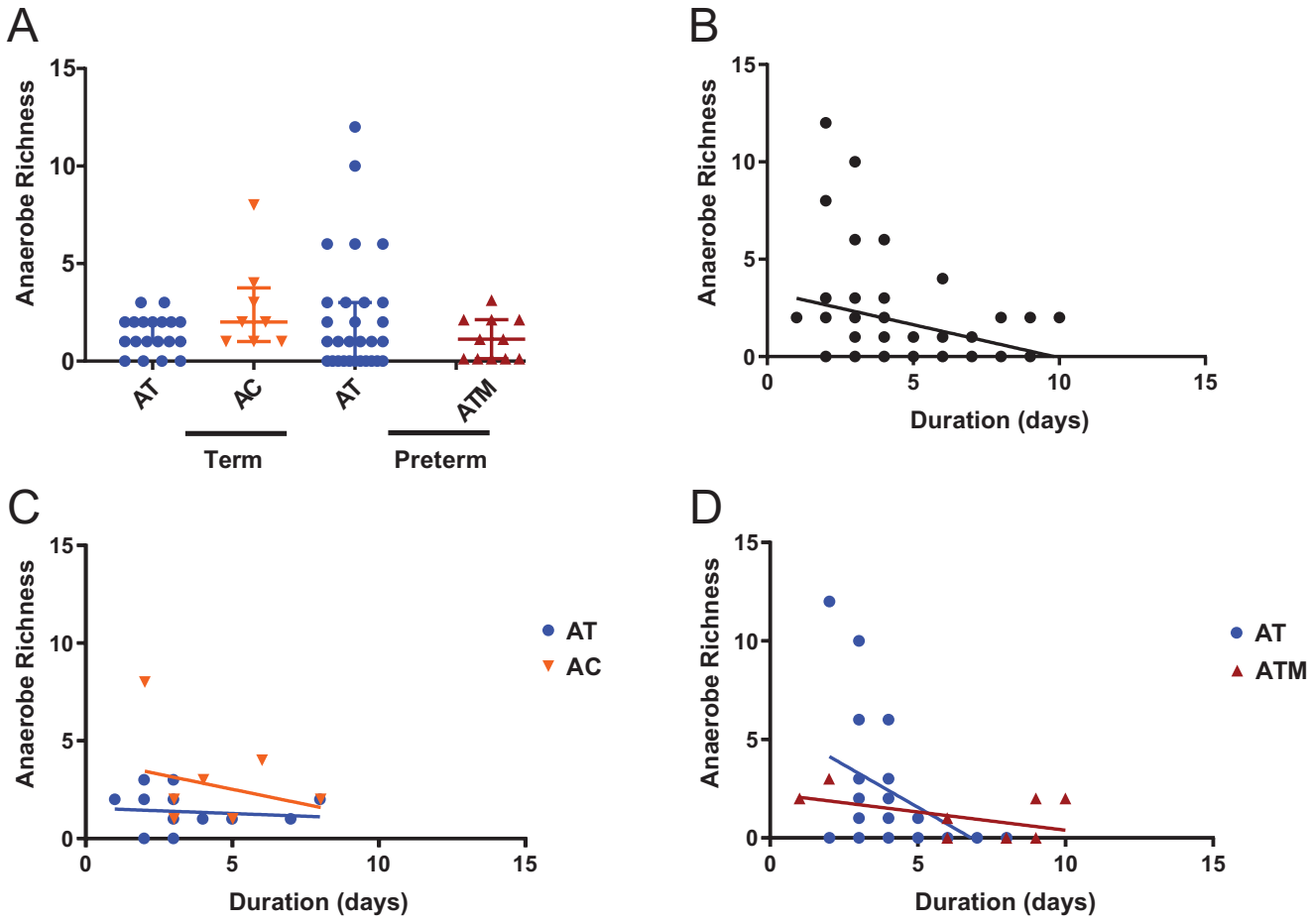

Figure 2. *A*, Obligate anaerobe richness (number of unique taxa at the genus level) in term and preterm infants, stratified by treatment regimen received: AT, AC, or ATM. Median and interquartile range are plotted. *B–D*, Simple linear regression of duration of therapy, measured in days, on the richness of obligate anaerobes in all infants (*B*) and in term (*C*) and preterm (*D*) neonatal intensive care unit patients by treatment regimen received. Patients with no bacterial 16s rRNA gene density, as determined by the quantitative polymerase chain reaction cutoff for detectable bacterial density, were included as zeroes in the measures of anaerobe richness. Abbreviations: AC, ampicillin and cefotaxime; AT, ampicillin and tobramycin; ATM, ampicillin, tobramycin, and metronidazole.

have captured prior regimens, especially in older infants who were admitted to our NICU from other hospitals. AC therapy was only prescribed to term infants, while infants with GI/abdominal indications were prescribed longer durations of metronidazole compared to the other indications. Although we controlled for these confounders, they are potentially associated with additional unmeasured confounders of gut microbiota composition. The 16S rRNA gene sequencing has limited taxonomic resolution, which restricted our ability to infer the anaerobic status of all taxa. Shotgun metagenomic sequencing would enable more precise taxonomic annotation.

Our cohort did not include either repeat post-treatment sampling or linking to in-patient or longitudinal outcomes. Although infancy represents a critical developmental window for the microbiota, the microbiota may recover between different treatment groups equally regardless of antimicrobial exposure. However, antimicrobial exposure in this period in humans [3, 4, 6, 7] and microbiota perturbation during a critical time window (2–4 weeks) in mice has been linked to long-term outcomes [37].

We were not powered to analyze duration-specific differences during different time windows (eg, first days vs later days after initiation of antibiotics). Most antibiotic use in the NICU is empiric, initiated for suspected rather than proven infection, and is common for culture-negative sepsis rather than culture-proven sepsis in neonates [1, 2]. Antimicrobial duration increases the risks of *C. difficile* [38] or antimicrobial resistance [39] in as little as 24–48 hours, and it is possible that the greatest risk to the microbiota may occur in the initial few days of therapy.

In conclusion, we found that in all infants, each additional day of antibiotics was associated with lower obligate anaerobes and butyrate-producers. Our findings add to the expanding literature on the unintended harms associated with prolonged antibiotic use in neonates. This information may be incorporated into risk/benefit assessments for discontinuing antibiotic therapy where continuation is inappropriate. Further research

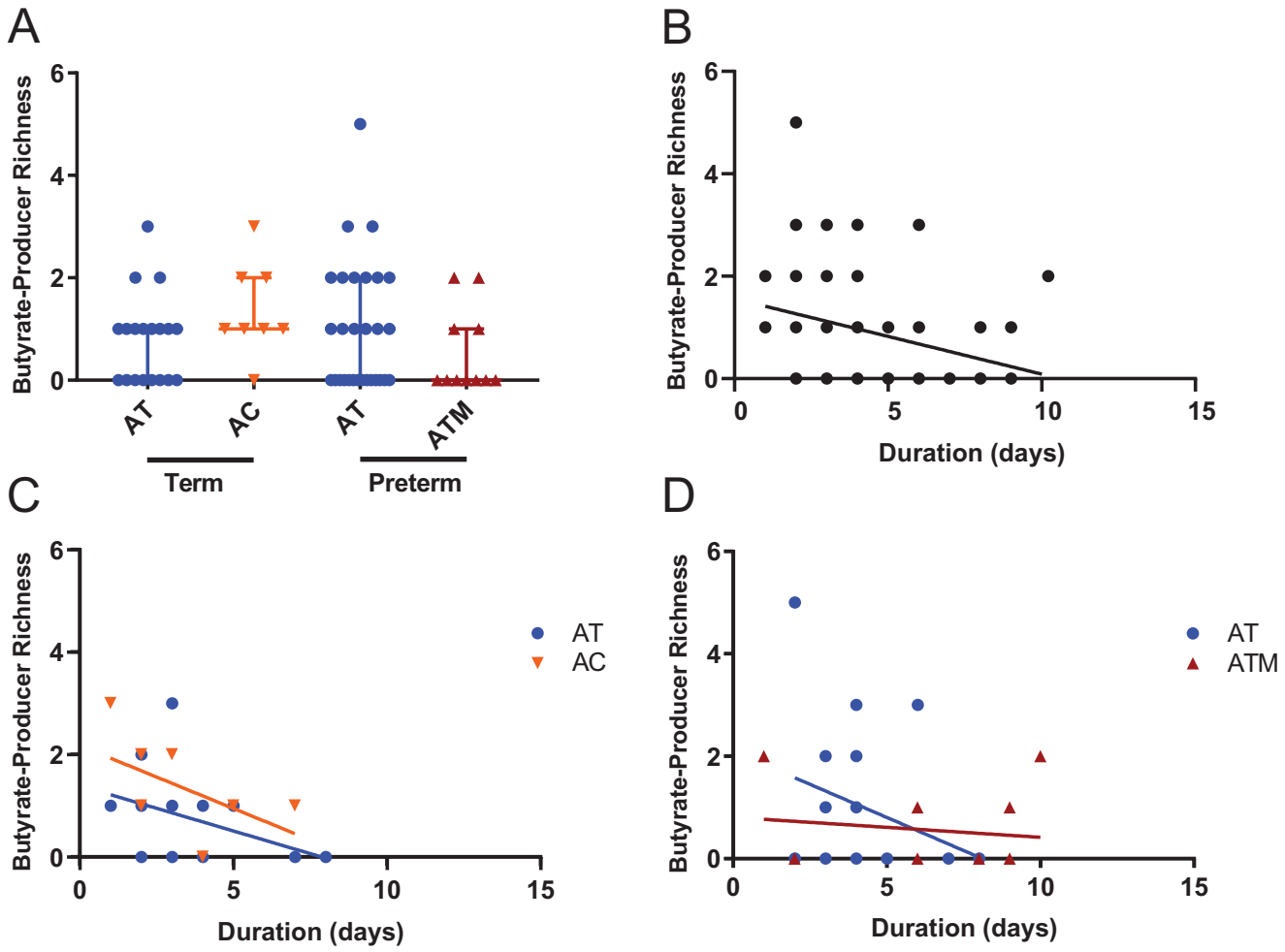

Figure 3. *A*, Butyrate-producer richness (number of unique taxa at the family level) in term and preterm infants, stratified by treatment regimen received: AT, AC, or ATM. Median and interquartile range are plotted. *B–D*, Simple linear regression of duration of therapy, measured in days, on the richness of butyrate-producers in all infants (*B*) and in term (*C*) and preterm (*D*) neonatal intensive care unit patients by treatment regimen received. Patients with no bacterial 16s rRNA gene density, as determined by the quantitative polymerase chain reaction cutoff for detectable bacterial density, were included as zeroes in the measures of butyrate-producer richness. Abbreviations: AC, ampicillin and cefotaxime; AT, ampicillin and tobramycin; ATM, ampicillin, tobramycin, and metronidazole.

is needed to determine whether incorporation of microbiome information into therapeutic decision-making will impact prescribing practices or improve clinical outcomes. When prolonged therapy is needed to treat infection, coadministration or postantimicrobial pre- and/or probiotics may represent a strategy to restore or protect health-associated anaerobes and

Table 2. Crude and Adjusted Effect Estimates of Antimicrobial Regimen Variables on the Richness of Anaerobes and Butyrate-producers

	Obligate Anaerobe Richness		Butyrate-producer Richness	
Independent Variable	cRR (95% CI)	aRR (95% CI)[a]	cRR (95% CI)	aRR (95% CI)[a]
Antibiotic duration[b]	0.83 (.72–.94)[c]	0.84 (.73–.95)[d]	0.82 (.70–.95)[e]	0.82 (.67–.97)[f]
ATM vs AT	0.60 (.26–1.36)	0.81 (.27–2.62)	0.71 (.27–1.57)	1.23 (.29–6.56)
AC vs AT	1.72 (.80–3.86)	2.11 (1.07–4.10)[e]	1.68 (.76–3.33)	1.86 (.76–4.24)

Abbreviations: AC, ampicillin and cefotaxime; aRR, adjusted risk ratio; AT, ampicillin and tobramycin; ATM, ampicillin, tobramycin, and metronidazole; cRR, crude risk ratio; CI, confidence interval.

[a]Adjusted for gestational age (weeks), age at sampling (days), time since antibiotic cessation (days), indication, mother's breast milk exposure, and delivery mode.

[b]Antibiotic duration is measured in days.

[c]*P* value is significant (*P* = .005).

[d]*P* value is significant (*P* = .008).

[e]*P* value is significant (*P* = .01).

[f]*P* value is significant (*P* = .03).

mitigate potentially harmful off-target effects of antimicrobials on the microbiota. Further research on safety and optimal agent is needed, and a large longitudinally sampled cohort is needed to validate the results of this study.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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