ORIGINAL

Influences of ampicillin exposure in early life on the murine gut microbiota and steatotic liver disease associated with western diet

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Abstract : Dysbiosis of gut microbiota is one of the important factors associated with metabolic dysfunction-associated steatotic liver disease (MASLD). Antibiotic use, especially in early life, could profoundly disrupt an establishing process of stable gut microbiota, and the influence on gut environment may persist throughout life. In this study, we examined effects of ampicillin exposure (AMP) in early life on the temporal changes of fecal microbiota and severity of MASLD in western diet-fed C57BL/6J mice. Histological evaluation of MASLD showed that steatosis in female mice and lobular inflammation was significantly influenced with AMP, and that NAS (MASLD activity score constituting from score of steatosis, lobular inflammation, and ballooning degeneration) tended to be high in female of AMP-treated group. 16S metagenome analyses of fecal microbiota showed significant decrease of α-diversity and remarkable shift to normally minor bacterial species at 4 weeks of age in AMP-treated mice, and the influence was continuously observed even after finishing the western diet feeding period. α-Diversity at 4weeks of age negatively correlated with combined scores of steatohepatitis and fibrosis. These results suggest that AMP in early life induced dysbiosis of gut microbiota and could promote the development of western diet-associated steatotic liver disease. J. Med. Invest. 73 : 186-207, February, 2026

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INTRODUCTION

The intestinal tracts of humans and other higher animals are home to a wide variety of symbiotic microorganisms that outnumber the host cells (1, 2). From immediately after birth, gut microbiota starts to be shaped by microbes inherited from the mother. At first, facultative anaerobes and aerobes colonize and make an anaerobic environment in infant's gut to help sequential colonization of strict anaerobes such as *Bifidobacterium, Bacteroides and Clostridium*, which are popular inhabitants in adult gut. During early life until weaning, infant's gut microbiome dynamically changed under influences of the host's genetic background and of peri- and post-natal factors such as delivery mode, diet, and antibiotic exposure. Host-microbe interactions and microbe-microbe interactions also affect the gut microbiota composition, resulting in establishment of a diverse and stable microbial community (1, 3-5). Structure of gut microbiota have been known to become like that of adult until approximately 3 years of age in human, while in mice it lasts until around 3–4 weeks of age (3, 6, 7) and once established microbiota could remain highly stable in healthy adults. Juan Miguel Rodríguez *et al*. (8) have emphasized an importance of increasing diversity of gut microbiota over the course of several years from birth, which could contribute to subsequent stability of gut microbiota throughout the life. Proper host–microbe interactions during this critical period are essential for establishing a diverse and plastic gut microbiota (3-5, 9).

Gut microbiota is closely associated with overall health status. It plays crucial roles in host intestinal barrier function, immune and metabolic homeostasis and disturbance of gut microbiota composition have been known to influence the risk of various diseases (1, 10, 11-13). Colonization of gut microbes strengthen intestinal barrier function through increasing expression of intercellular tight junction proteins and increasing secretion of mucin and antimicrobial substances in epithelial cells, and crosstalk between gut microbe and the host cells also contribute to regulate excessive host immune responses (14). Moreover, gut microbiota possesses metabolic pathways distinct from humans, producing organic acids such as acetate and butyrate from indigestible dietary components, and participating in bile acid metabolism. These microbial metabolites are absorbed and distributed throughout the body, exerting profound effects on host physiology (1, 11, 15, 16).

In contrast to healthy state, dysbiosis of gut microbiota characterized by reduced diversity and compositional shifts are known in patients with various non-communicable diseases (1,5, 10). Intervention with dietary fiber and specific nutrients have been reported to reduce disease risk via modulation of the gut environment (17). Gut microbes and their metabolites influenced the expression of barrier-related molecules, hepatic lipid metabolism, and cellular functions (11). Therefore, disruption of gut microbiota composition and its metabolites could continuously promote the development of a wide range of disorders, including lifestyle-related metabolic diseases. Metabolic

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dysfunction-associated steatotic liver disease (MASLD, formerly NAFLD) is one of the lifestyle-related diseases and is characterized by the accumulation of triglycerides in the liver, leading to hepatic injury (13). MASLD may sometimes be accompanied by inflammation and degeneration, which are termed metabolic dysfunction-associated steatohepatitis (MASH, formerly NASH), which may progress to cirrhosis and hepatocellular carcinoma. The liver is highly susceptible to microbial components and their metabolites from the gut via the portal vein, and many studies have suggested contributions of gut microbiota in the pathogenesis of fatty liver disease (1, 11, 13, 18-21). High-fat diets themselves alter the gut microbiota (12), and these changes, along with microbial metabolites, are thought to contribute to the onset and progression of steatohepatitis (13). Difference of basic structure, stability and resilience of gut microbiota are present among individuals, and such differences probably affect the responses of gut microbiota to high-fat diet and development of fatty liver disease (18-21).

Antibiotic use is one of the most significant factors influencing the gut environment (22, 23). While gut microbiota is relatively stable in adults, antibiotic exposure during early life could profoundly disrupt the gut environment, with effects that may persist throughout life (5, 6, 23-26). As described above, proper host–microbe and microbe-microbe interactions before the establishment of microbial community, are considered essential for establishing a diverse and resilient gut microbiota (9). Disruption or dysbiosis during this critical period may have long-term effects on microbial composition and host physiological functions, potentially contributing to earlier onset and exacerbation of lifestyle related diseases such as steatohepatitis.

In the present study, we intervened in gut microbiota from birth to weaning by *ad libitum* administration of ampicillin in drinking water, and then, mice were fed western diet from 8 to 28 weeks of age. We analyzed the effects of ampicillin exposure on onset and severity of steatotic liver disease, temporal changes in gut microbiota composition, and alterations in microbiota-derived organic acids. We further investigated the relationship between ampicillin-induced dysbiosis in gut microbiota and the onset/development of MASLD.

MATERIALS AND METHODS

Materials

C57BL/6J mice (six males and six females, all 8–10 weeks old) was purchased from Jackson Laboratory Japan, Inc. (Yokohama, Japan). Two types of rodent diets were used in this study : MFG, a normal chow diet (Oriental Yeast Co. Ltd., Tokyo, Japan, 359 kcal/100 g) ; Western diet (D12079B, Research Diets, Inc., New Brunswick, NJ, USA, 468.6 kcal/100 g). The latter contains 21% fat, 34% sucrose, and 0.15% cholesterol, and is commonly used to induce obesity-related insulin resistance leading to hepatic steatosis and steatohepatitis (27). Ampicillin sodium (Fujifilm Wako Pure Chemical Corporation, Tokyo, Japan) was used for early-life antibiotic exposure. For the analysis of the gut microbiota in mouse feces, molecular biology-grade reagents and sequencing reagents from Illumina Singapore Pte. Ltd. (Singapore, Singapore) were used as described below.

Animal treatment in western diet-induced NASH model

Animal experiments were conducted as described in Fig 1 in accordance with the Guidelines for Animal Experiments of Tokushima University under approval by the Animal Experiment Committee of Tokushima University (Approval No. T2021-103). C57BL/6J mice (both sexes) were acclimated to the housing environment, mated at 10–12 weeks of age, and pregnant females were transferred to individual cages before delivery. Offspring from 5 mother mice (both sexes) were used for three experimental groups : negative control (NC) group, positive control (PC) group, and ampicillin exposure (AMP) -treated group (AMP-treated -I, -II, -III). The NC group (3 males, 3 females, littermate from a mother No.1) was fed a normal chow diet from weaning until the end of the experiment. The PC group (3 males, 4 females, littermate from a mother No.2) was allowed *ad libitum* access to the western diet from 8 to 28 weeks of age. The AMP-treated group (11 males, 9 females, littermates from 3 mother mice No.3, 4, 5) was exposed to 1 g/L ampicillin sodium as drinking water from birth to 3 weeks of age as described in Castro-Mejía JL *et al*. (28). Ampicillin solution was provided to the dams from 1–2 days prior to delivery, and offsprings were exposed to ampicillin until weaning while housed together with

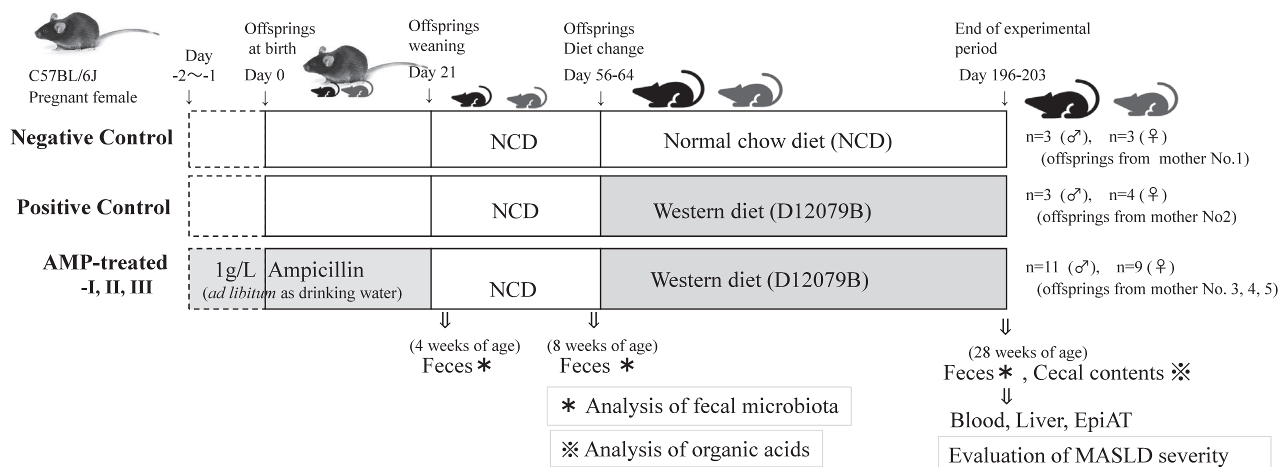


Figure 1. Experimental schedule. Offsprings from 5 mother mice were used for three experimental groups and treated as described in Material & Methods. Negative control group from a mother (No.1) was not exposed to ampicillin and fed normal chow diet (NCD) throughout the experimental period. Positive control group from a mother (No.2) was fed western diet from 8 to 28 weeks of age. AMP-treated group (-I, II, III) from three mothers (No.3, 4, 5) were exposed to ampicillin until weaning and were fed western diet in the same way as positive control group.

their mothers. Ampicillin solution was replaced to fresh one every 2–3 days. After weaning 3 weeks of age, mice were maintained on a normal chow diet, and they were fed the western diet from 8 weeks of age for 20 weeks like in PC group. Body weight was measured weekly from 4 weeks of age. Feces (about ~50mg) were collected at 4, 8, and 28 weeks of age for analysis of fecal microbiota. Fecal samples were washed with TE buffer, resuspended at 0.1g feces/ml to prepare Gram-stained specimens (10μl/cm^2) for preliminary observation of microbiota changes. Fecal suspensions were centrifuged again, and the sediments were stored at –30 °C until analysis of the gut microbiota.

At the end of experimental period, under anesthetizing with isoflurane, whole blood samples were obtained via orbital venous puncture, and mice were subsequently euthanized by cervical dislocation. Liver, epididymal adipose tissue (EpiAT) were resected and weighed. Portions of the liver were fixed in formalin, embedded in paraffin, and processed for evaluation of severity of steatotic liver disease. Cecal contents were collected and stored at –30 °C until analysis of organic acids produced by gut microbiota. Collected blood was allowed to clot at room temperature for approximately 30 minutes, followed by centrifugation to separate serum, which was stored at –30 °C for analyses of hepatopathy biomarker. In case of body weight loss exceeding 20% or persisted for more than four weeks, humane endpoints were applied on the mice and samples were collected even before the end of experiment.

Evaluation of severity of steatotic liver disease

The severity of steatotic liver disease was evaluated by measuring serum liver injury markers and histological assessment on the liver tissue sections. Serum collected at the end of the experiment was sent to Nagahama Life Science Laboratory, Oriental Yeast Co., Ltd. (Nagahama, Japan) for measurement of ALT (standardized JSCC method) and total bile acids (TBA, enzymatic cycling method). Histological evaluation for steatohepatitis and fibrosis was performed according to the scoring system in Kleiner *et al*. (29). Steatosis (distribution of lipid droplets, grade 0-3), lobular inflammation (grade 0-3), and ballooning degeneration (grade 0-2) were assessed on HE-stained liver sections and sum of the three scores constituted the MASLD activity score (NAS). NAS values ≥ 5 was judged to be onset of steatohepatitis. The degree of fibrosis (stage 0-4) was evaluated on serial sections, which were stained with Sirius Red–Fast Green Collagen staining kit (Chondrex, Inc., USA, cat.no. 9046) according to the manufacturer's instruction.

Fecal microbiota analysis

Detailed analysis of fecal microbiota composition at 4, 8, and 28 weeks of age was performed using 16S metagenomic sequencing as described below. Bacterial genomic DNA was extracted according to the method of Morita H *et al*. (30). Frozen fecal samples were thawed and treated with lysozyme and achromopeptidase (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), followed by proteinase K (Nacalai Tesque, Inc., Kyoto, Japan) and SDS, and then DNA was extracted and purified by phenol–chloroform method. Quality and quantity of DNA was checked by Nanodrop 2000 (Thermo Fisher Scientific, Inc., MA, USA) and agarose gel electrophoresis.

Sequencing libraries were prepared as described in the Illumina 16S Metagenomic Sequencing Library Preparation protocol (15044223 Rev. B JPN). The V3–V4 region of 16S rDNA was amplified, and a second PCR was performed with Nextera XT index kit v2 Set A (cat.no. 15052163, Illumina, Inc.), following purification with AMPure XP beads (Beckman Coulter, Inc.). The integrity of the DNA amplicon was checked by agarose gel electrophoresis. The loading DNA concentration was adjusted to 4pM each using a Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Inc.). The libraries were spiked with 25% PhiX control v3, sequenced on a MiSeq system (Illumina, Inc.) with MiSeq® Reagent Kit v3 (cat.no. MS-102-3003, 600 cycles, Illumina, Inc.) to generate paired-end reads of 300 bases in length in each direction. Operational taxonomic unit (OTU) clustering of the sequencing reads was performed with Qiime2 with the UCLUST algorithm at 97% similarity against the Greengenes reference database (v.13.8). In total, 12,998,440 reads with an average of 135,400 reads per sample were obtained.

Microbiome analyses were performed using the phyloseq package (v.1.50.0) in R (v.4.4.2). Calculation of α-diversity indices was performed using the MicrobiotaProcess package. β-diversity (Bray–Curtis distances) among samples was calculated with the vegan R package (v.2.6-10) based on the relative abundances of observed OTUs. Clustering of the samples was visualized by performing a principal coordinates analysis (PCoA) using the Bray–Curtis distance metric. Differential analyses of OTU abundances were performed with linear discriminant analysis effect size (LEfSe) implemented in the MicrobiotaProcess package.

Quantification of Bifidobacterium

To examine the influence of AMP exposure on the number of genus *Bifidobacterium* at 4, 8, and 28 weeks of age, genus-specific quantitative PCR was performed as described by Matsuki T *et al*. (31) using primers g-Bifido-F (5'-CTC CTG GAA ACG GGT GG-3') and g-Bifido-R (5'-GGT GTT CTT CCC GAT ATC TAC A-3'). Reactions were performed with Fast SYBR™ Green Master Mix (Life Technologies Japan Ltd., Tokyo, Japan, cat.no. 4385612) on a QuantStudio® 3 Real-Time PCR System (Thermo Fisher Scientific K.K.), using cycling conditions : 95℃ for 30 s, followed by 50 cycles of 95℃ for 5 s, 55℃ for 30 s, and final extension at 72℃ for 30 s, with melt curve analysis. *Bifidobacterium longum* JCM 1217 (10^7 cells/tube) was used as a standard. Its DNA was extracted in the same way as for fecal samples. Serially diluted standard DNA was simultaneously assayed to calibrate the amount of *Bifidobacterium* in fecal samples. The number of *Bifidobacterium* cells per gram of feces was calculated as : Cell count (cells/g) = Quantity Mean × Dilution Factor/Fecal weight for DNA extract (g)

Analysis of organic acids in cecal contents

Organic acid composition in cecum contents at 28 weeks of age was analyzed by Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan). Following nine organic acids were quantified using high-performance liquid chromatography with a Shimadzu organic acid analysis system : succinate, lactate, formate, acetate, propionate, *iso*-butyrate, *n*-butyrate, *iso*-valerate, and *n*-valerate. The lower limit of quantification was 0.05 mg/g for succinate, lactate, acetate, and propionate, and 0.1 mg/g for the other acids.

Statistical analysis

To explore the features of gut microbiota which are strongly associated with the severity of steatotic liver disease, machine learning approaches were employed. At first, mice were classified into three groups based on their NAS : High (≥5), Middle (4), and Low (0–3), and random forests classification model (32) was built for prediction of severity of MASLD as a function of microbiota features such as diversity indices and the compositions at phylum level (limited to phylum due to small sample size) from the 16S metagenomic data at 4, 8, and 28 weeks of age. Important features of microbiota to predict High or Low NAS group were calculated and confirmed by feature selection with the Borta Package (33).

The data were statistically analyzed by using Excel Statistics,

Statcel 3. Kruskal-Wallis test and/or Steel–Dwass test for comparisons among multiple groups, and Welch's t-test (when variances were unequal) or Mann–Whitney U test (for non-parametric data) for comparisons between two groups. Correlations between features of gut microbiota composition and histological severity of steatotic liver disease were analyzed using Spearman's correlation coefficient by rank test. Differences with p-values < 0.05 were considered statistically significant.

RESULTS

Body weight changes, organ weights, and serum markers in mice

According to the experimental schedule in Fig 1, offspring mice were treated with or without AMP from birth to 3 weeks of age and fed normal chow diet (NCD) until 8 weeks of age. After collecting feces at 8 weeks of age, diet for PC group and AMP-treated group was changed to western diet. During the experimental period from 8 to 28 weeks of age, the average of total caloric intake (Kcal/mouse) in male mice was 1445.4 in negative control maintained on normal chow diet, 1769.0 in the positive control fed western diet, and 1900.1 in the AMP-treated group fed western diet, while in females it was 1395.5, 1638.0, and 1572.5, respectively. Total caloric intakes were higher in males than in females across all groups. The total ampicillin exposure per cage in early life was estimated based on water consumption to be 273.4 mg (AMP-treated-I, 4 pups), 396.6 mg (AMP-treated-II, 7 pups), and 430.2 mg (AMP-treated-III, 9 pups).

Table 1 summarized the parameters at the end of experimental period. Western diet-fed groups, PC group and AMP-treated group showed significantly greater body weight increase (%) during 8-28 weeks of age compared to NC group. The weight gain in female mice was less pronounced than that in males. Body weight in AMP-treated group was slightly lower at 4–5 weeks of age but became comparable to those in the positive control from 6 weeks of age. Western diet significantly increased EpiAT and the liver weight with a greater increase in males, compared to NC group. Livers in the western diet-fed groups macroscopically showed steatotic features. While no significant influences of ampicillin exposure were found on organs weight, 3 mice in AMP-treated group (1 male in AMP-treated-II, 2 females in AMP-treated-III) showed liver atrophy. Serum ALT and TBA, biomarkers of liver injury, tend to be high in western diet-fed mice compared to NC group. AMP-treated group in both sexes showed a significantly elevated ALT value, although the difference was not statistically significant in male mice of the PC group due to inter-individual variation. Fasting blood glucose at 28 weeks of age also showed a similar trend. Serum TBA level was not significantly different among the groups, but mice with macroscopic liver atrophy in AMP-treated group had elevated values. Two female mice in NC group were excluded from the following analyses because of the abnormally high TBA value and abnormal liver tissue finding. These results indicate that western diet from 8 weeks of age induced obesity-associated steatotic liver disease, although consistent statistical difference with AMP in early life was not detected in these parameters in either sex.

Histological evaluation of steatohepatitis

Histological severity of MASLD was microscopically evaluated on mouse liver sections. Fig. 2 a, b, c shows representative micrographs of the HE-stained liver tissues. Table 2 presents grade of steatosis, lobular inflammation and ballooning degeneration, and the sum of these scores, NAS, in each mouse. In male mice, steatosis was prominent in western diet-fed groups. Individuals with NAS ≥5, indicative of steatohepatitis, appeared in male of both the PC group and AMP-treated group. In female mice, steatosis grade in PC group was lower than in males and the NAS remained below 5. However, steatosis grade in female of AMP-treated group was significantly higher than PC group, and NAS tend to be high with two individuals having developed MASH. Differences attributable to AMP treatment were also detected in lobular inflammation level between NC group and AMP-treated group. Fibrosis (0–4) was assessed on Sirius Red–Fast Green–stained sections, with red-stained regions indicating fibrosis, using the negative control group as baseline (Fig. 2 d, e). Fibrosis stage significantly correlated with serum TBA level (Spearman's rank correlation coefficient = 0.511, $p < 0.001$), supporting the validity of histological severity results. The severity of fibrosis was individually varied in both male and female mice, but in AMP-treated group, individuals with macroscopic liver atrophy exhibited higher fibrosis stage. Although

Table 1. Body weight increase, adipose tissue weight, liver weight, and serum biochemical parameters in male and female mice at the end of experimental period.

(Male mice)

	Ampicillin exposure in early life	Diet for 8-28 weeks of age	mice number	Body weight increase (%) for 8-28 weeks of age	EpiAT (g)	Liver weight (g)	Fasting blood glucose (mg/dl)	Serum ALT (IU/ml)	Serum TBA (μmol/l)
Negative Control	(-)	NCD	3	130.4 ± 3.7	1.0 ± 0.3	1.3 ± 0.2	94.7 ± 12.9	23.7 ± 3.2	7.7 ± 3.1
Positive Control	(-)	Western diet	3	195.5 ± 15.5**	2.4 ± 0.1**	3.1 ± 0.6**	126.0 ± 17.7	299.7 ± 189.5	23.0 ± 18.1
AMP-treated	(+)	Western diet	11	191.3 ± 21.7**	2.3 ± 0.5**	2.7 ± 0.7**	145.5 ± 21.8**	188.8 ± 75.7**	20.8 ± 27.9

(Female mice)

	Ampicillin exposure in early life	Diet for 8-28 weeks of age	mice number	Body weight increase (%) for 8-28 weeks of age	Liver weight (g)	Fasting blood glucose (mg/dl)	Serum ALT (IU/ml)	Serum TBA (μmol/l)
Negative Control	(-)	NCD	3	124.6 ± 4.7	0.9 ± 0.2	81.0 ± 8.7	20.0 ± 3.6	20 (n=1)
Positive Control	(-)	Western diet	4	148.6 ± 9.0*	1.3 ± 0.2*	126.5 ± 29.4	50.0 ± 8.6*	8.5 ± 2.9
AMP-treated	(+)	Western diet	9	159.8 ± 17.7*	1.5 ± 0.3*	123.8 ± 22.0*	65.0 ± 23.6*	46.8 ± 69.7

Values are Mean±SD.
Significantly different from negative control group. *p<0.05, **p<0.001 (Welch's t-test)

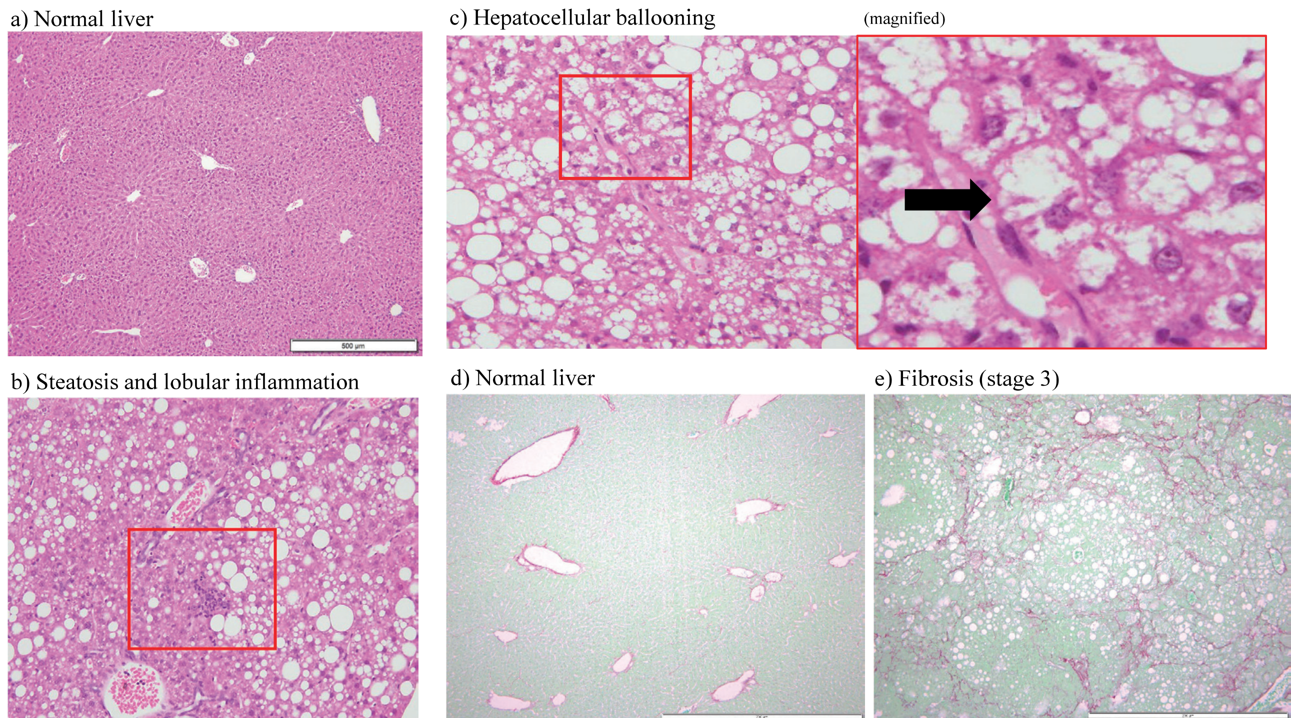


Figure 2. Representative micrographs of the liver tissues of mice at the end of experimental period. a) Normal liver of negative control group (×100 magnification; HE stained). b) Steatosis and lobular inflammation, and c) Ballooning degeneration in the liver of AMP-treated group (×200 ; HE stained). d) Normal liver of negative control group and e) Fibrosis in the liver of AMP-treated group (×200 ; stained with Sirius Red-Fast Green staining kit).

differences between PC group and AMP-treated females were not statistically significant overall, the occurrence of more severe MASH in some AMP-treated mice with dependent on the difference of mother mouse suggests that early-life antibiotic exposure may predispose to onset and/or development of MASLD through interfering with gut microbiota developing process.

Fecal microbiota analysis (16S metagenomic analysis)

A total of 96 fecal samples collected at 4, 8, and 28 weeks of age were subjected to 16S metagenomic sequencing to analyze microbiota composition. Fig. 3a and Table S1 shows the relative abundances of 65 major genera comprising the fecal microbiota of individual mice in each experimental group at 4, 8, and 28 weeks. Early-life AMP, from birth until 3 weeks of age, had a profound impact on the fecal microbiota at 4 weeks of age, with a clear reduction in the number of constitutive bacterial taxa. All groups ate normal chow diet after weaning, and the impact of AMP became less at 8 weeks of age. At 28 weeks of age, the microbiota composition in western diet-fed groups (PC group, AMP-treated group) differed from those in NC group, while there was no significant decrease in the *Bacteroidetes*/*Firmicutes* ratio at the phylum level.

Richness of gut microbiota in early life is one of the important factors for stable gut microbiota. At 4 weeks of age, AMP treatment remarkably decreased α-diversity compared with untreated groups (NC, PC) in both Chao1-based and Shannon diversity (Fig. 3b). Significant decrease of α-diversity remained at 8 weeks of age in both male and female mice. At 28 weeks of age, western diet feeding for 20 weeks increased the α-diversity in the PC group, whereas the significantly lower diversity remained in the AMP-treated group despite the similar intake of western diet.

To see an effect of early life AMP on age-dependent change of fecal microbiota, β-diversity of the fecal microbiota was assessed by principal coordinate analysis (PCoA) based on Bray–Curtis distances of operational taxonomic units (Fig. 3c). At 4 weeks of age, fecal microbiota in AMP-treated mice clustered distinctly from unexposed NC and PC groups, indicating strong effects of early-life AMP on microbiota composition just after the treatment. Interestingly, among AMP-treated groups (AMP-treated-I, II, III) the microbiota clustered within each littermate, suggesting maternal influence on fecal microbiota in early stage. Difference of microbiota composition between AMP-treated and untreated groups was detected at 8 weeks of age. Western diet feeding greatly influenced on microbiota and western diet-fed groups (PC, AMP-treated) showed different compositions from NC group at 28 weeks of age. But AMP-treated groups exhibited notably distinct microbiota from PC group, suggesting that early-life AMP altered the trajectory of microbiota changes following western diet feeding.

To identify bacterial biomarkers differentiating the five groups (NC, PC, AMP-treated-I, II, III), LEfSe (Linear Discriminant Analysis Effect Size) analysis was performed (Fig. 3d). Bacterial taxa with significant difference (Kruskal-Wallis test, $p<0.05$) and with LDA scores > 2.0 were identified as biologically important biomarkers. At 4 weeks of age, g_*Granulicatella* and g_*Enterococcus* were enriched in AMP-treated III (accounting for 89-99% of the total), whereas g_*Bifidobacterium* and g_*Turicibacter* were characteristic of another antibiotic-exposed group AMP-treated I (47-91% of the total). Although relative abundance was very low, g_*Staphylococcus* in AMP-treated I, g_*Dehalobacterium* and g_*Clostridium* in PC, were also characteristic. Thus, early-life AMP shifted the microbiota composition toward dominance by a few genera, consistently with the reduced α-diversity (Fig. 3b). However, dominant genera in AMP-treated groups differed across mother mice : *Enterococcus* and *Granulicatella* were predominant in AMP- II, III, whereas *Bifidobacterium* was more

Table 2. Evaluation of histological severity of MASLD in individual mouse.

Group	Treatment			MASLD activity								Fibrosis	
	AMP in early life	Diet from 8 weeks of age		Steatosis	Mean ± SD (Steatosis)	Lobular inflammation	Mean ± SD (Lobular inflammation)	Balloning degeneration	Mean ± SD (Ballooning degeneration)	NAS	Mean ± SD (NAS)	Fibrosis	Mean ± SD (NAS)
Negative control	(-)	Normal chow diet	NC-Male-1	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0 ± 0	0	0
			NC-Male-2	0		0		0		0		Sample, not enough	
			NC-Male-3	0		0		0		0		0	
			NC-Female-2	0	0	0	0	0	0	0	0	0	0
			Mean ± SD	0 ± 0		0 ± 0		0 ± 0		0 ± 0		0 ± 0	
Positive control	(-)	western diet D12079B	PC-Male-1	3	3 ± 0	1	1.33 ± 0.58	0	1.00 ± 1.00	4	5.33 ± 1.53	2	1.33 ± 1.15
			PC-Male-2	3		1		1		5		0	
			PC-Male-3	3		2		2		7		2	
			PC-Female-1	1	1 ± 0	0	1.00 ± 0.82	1	0.75 ± 0.96	2	2.75 ± 0.96	1	0.75 ± 0.50
			PC-Female-2	1		1		2		4		1	
			PC-Female-3	1		1		0		2		0	
			PC-Female-4	1		2		0		3		1	
			Mean ± SD	1.86 ± 1.07*		1.14 ± 0.69		0.86 ± 0.90		3.86 ± 1.77*		1.00 ± 0.82	
AMP-treated	(+)	western diet D12079B	AMP I - -Male-1	3	2.64 ± 0.81 ##	1	1.18 ± 0.60 ##	0	0.91 ± 0.83	4	4.73 ± 0.79 #	2	0.64 ± 1.03
			AMP II - Male-1	3		1		1		5		0	
			AMP II - Male-3	3		1		1		5		0	
			AMP II - Male-4	3		1		2		6		0	
			AMP II - Male-6	1		3		0		4		3	
			AMP III - Male-1	3		1		0		4		0	
			AMP III - Male-2	3		1		1		5		1	
			AMP III - Male-3	3		1		2		6		0	
			AMP III - Male-4	3		1		1		5		1	
			AMP III - Male-5	1		1		2		4		0	
			AMP III - Male-6	3		1		0		4		0	
			AMP I - Female-1	2	1.75 ± 0.71 §	1	1.38 ± 0.92	0	0.75 ± 0.89	3	3.88 ± 1.55	1	1.00 ± 1.31
			AMP I - Female-2	2		1		1		4		0	
			AMP I - Female-3	2		2		0		4		0	
			AMP II - Female-1	1		0		1		2		0	
			AMP II - Female-2	1		1		0		2		1	
			AMP II - Female-5	3		1		0		4		0	
			AMP III - Female-1	2		2		2		6		3	
			AMP III - Female-4	1		3		2		6		3	
			Mean ± SD	2.26 ± 0.87**		1.26 ± 0.73**		0.84 ± 0.83		4.36 ± 1.21**		0.79 ± 1.13	

Hisological data of one female in AMP-treated group (AMP III - F 2) was excluded because of death before sacrifice. Significant difference from male of negative control group, # $p<0.05$, ## $p<0.01$, Significant difference from whole negative control group, *$p<0.05$, **$p<0.01$ (Steel-Dwass test). Significant difference from female of positive control group, § $p<0.05$ (Welch's t- test).

Figure 3 a)

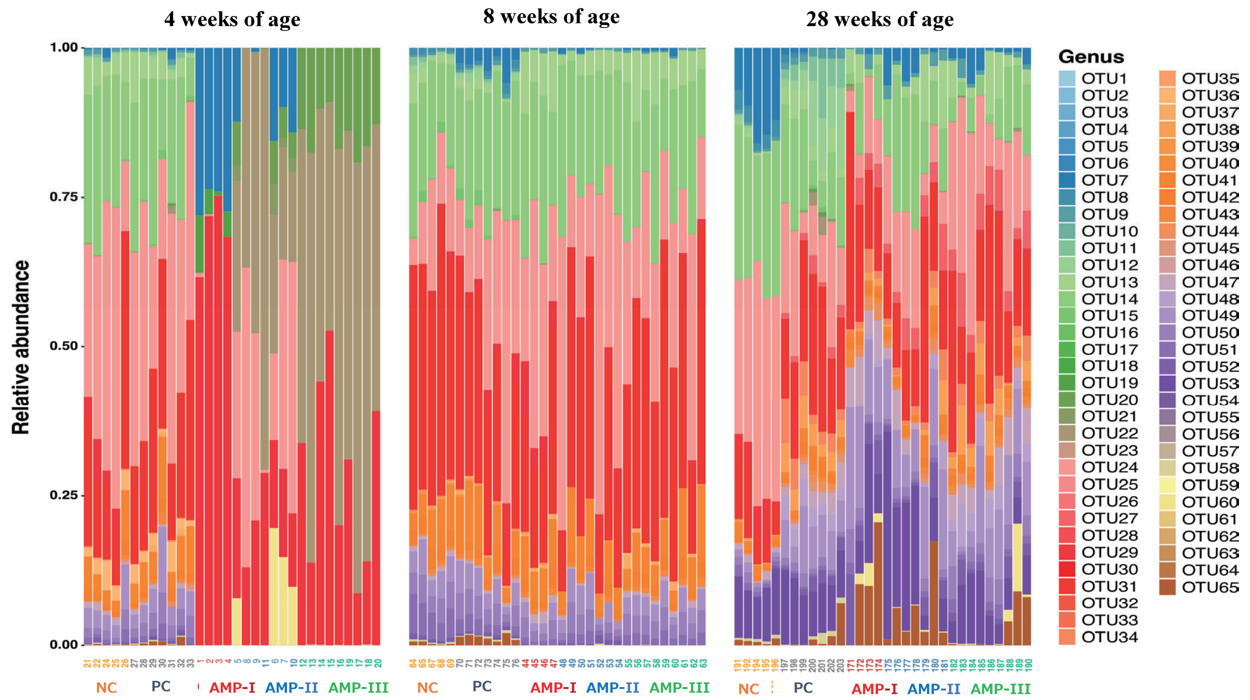
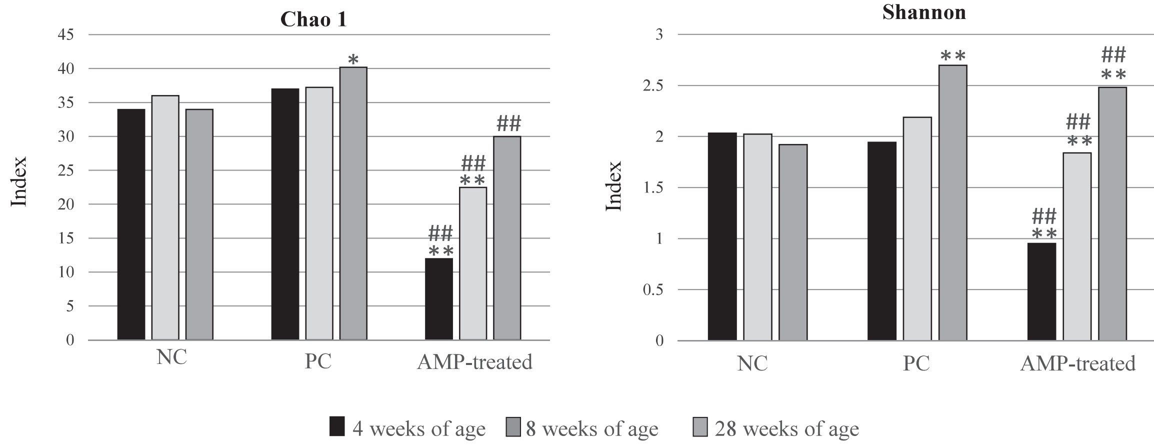


Figure 3 b)



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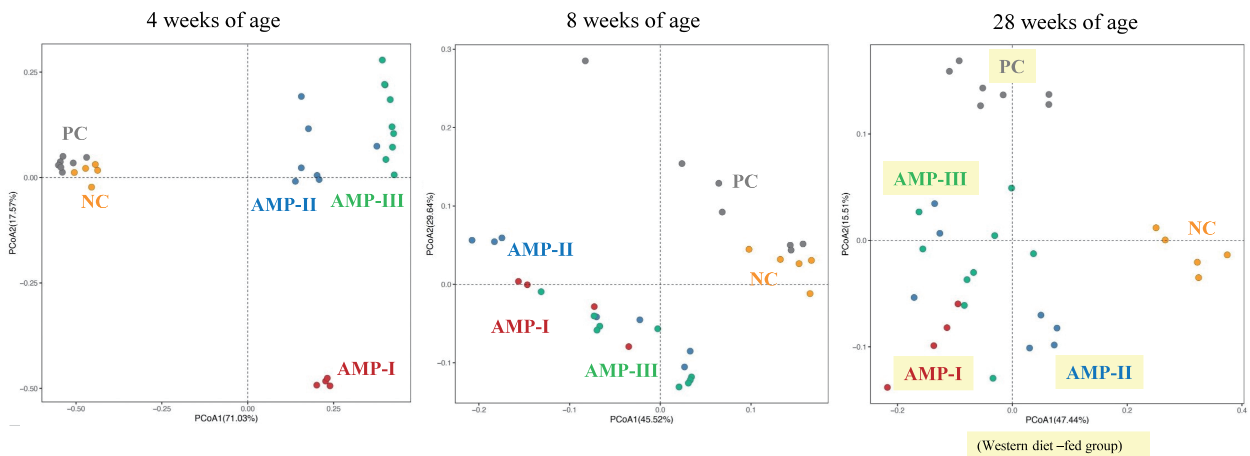

Figure 3 d)

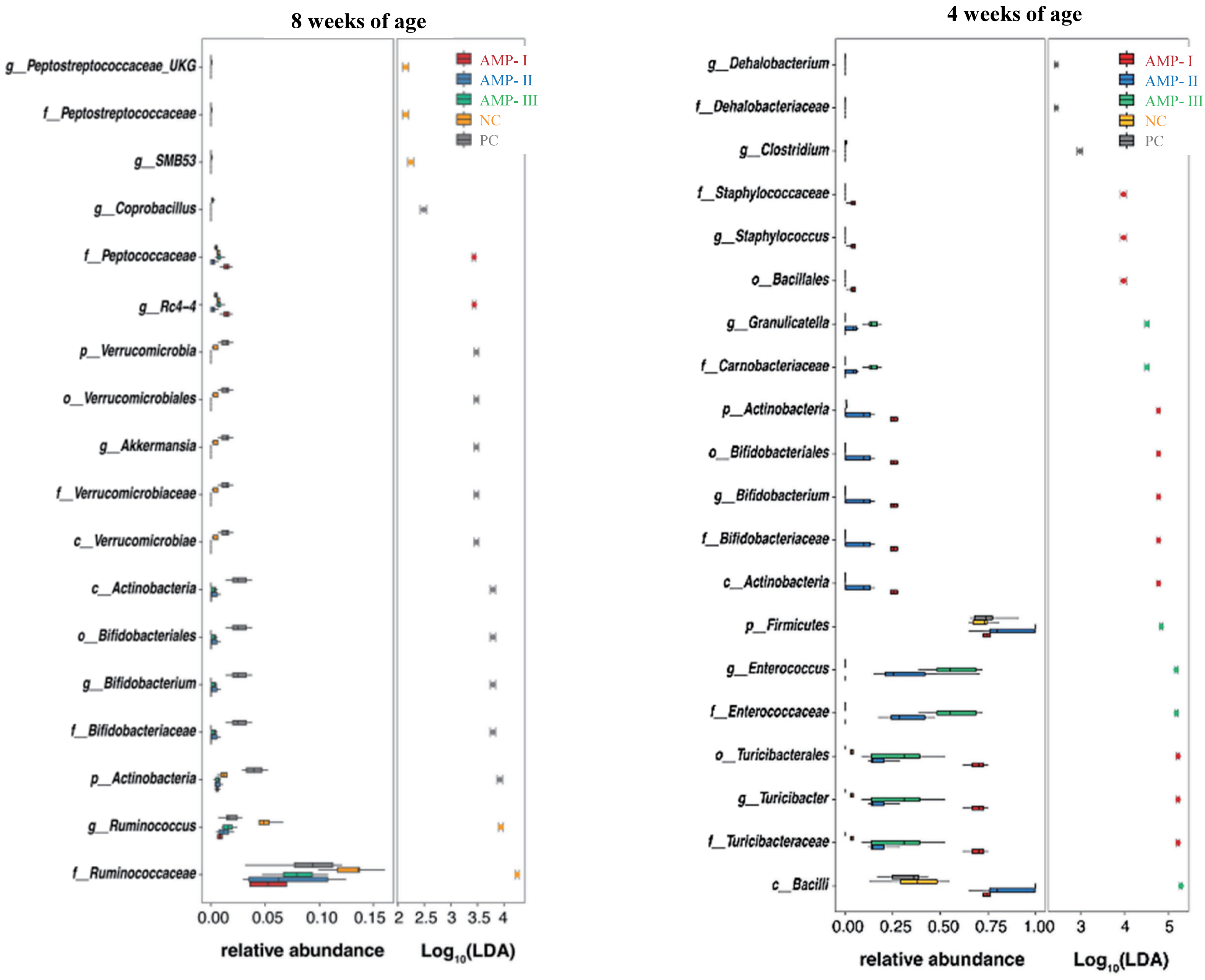

Figure 3 d)

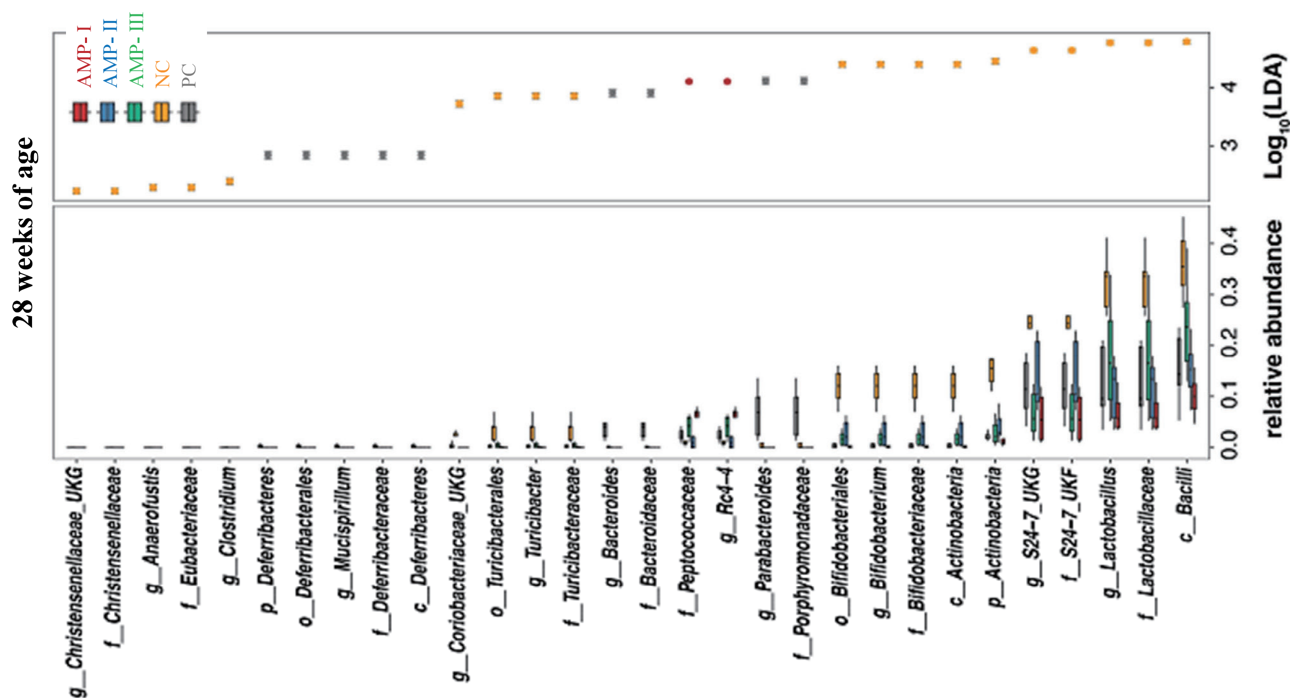

Figure 3. Effect of ampicillin exposure in early life and western diet feeding on fecal microbiota of mice at 4, 8, and 28 weeks of age. a) Relative abundance of main 65 genera. b) α-Diversity of the fecal microbiota in mice groups at each age. Values are median for each group at 4, 8 and 28 weeks of age. Significant difference from negative control (NC) group at the same age, *$p<0.05$, **$p<0.001$ (Mann-Whitney's U test). Significant difference from positive control (PC) group at the same age, $p < 0.05$, $p < 0.001$ (Mann-Whitney's U test). c) Principal coordinate analysis (PCoA) based on the Bray-Curtis distance of operational taxonomic units at 4, 8, and 28 weeks of age. β-Diversity of fecal microbiota composition was compared among the groups, negative control (NC), positive control (PC) and AMP-treated group (AMP-I, II, III, male and female mice born from mother No.3, 4, 5). d) Linear discriminant analysis effect size (LEfSe). Differential analyses of OTU abundances were performed as described in Material and Methods.

abundant in AMP-treated-I, II. At 8 weeks of age, characteristic genera were g_*Akkermansia*, g_*Bifidobacterium* and g_*Coprobacillus* in PC, g_*Ruminococcus* and g_SMB in NC, and g_Rc4-4 in AMP-treated-I. Influence of early-life AMP on fecal microbiota composition was found at this age, but the overall differences became less pronounced in parallel with partially recovered α-diversity. At 28 weeks of age, NC group were enriched in g_S24-7_UCG, g_*Bifidobacterium*, g_*Lactobacillus*, g_*Turicibacter* and g_*Coriobacteriaceae*_UCG, as well as less abundant in genera such as g *Christensenellaceae*_UCG, g_*Anaerofustis*, and g_*Clostridium*. In contrast, PC group fed western diet were enriched in g_*Bacteroides*, g_*Parabacteroides*, and g_*Mucispirillum*, a minor genus. While western diet feeding for 20 weeks markedly modulated the fecal microbiota composition in PC group, fecal microbiota in AMP-treated groups differently changed in response to western diet. In AMP-treated groups, g_Rc4-4 was enriched in AMP- treated-I, but relative abundance of the genera enriched in PC group did not increase. AMP in early life was found to modulate microbiota responses to western diet feeding. These results (Fig. 3a–d) demonstrate that early-life AMP exposure strongly influenced microbial diversity and composition of fecal microbiota at 4 weeks of age, with persisting into 8 weeks of age, and that western diet-responding alteration of microbiota composition was also modulated in early-life AMP- treated mice.

Abundance of Bifidobacterium

As *Bifidobacterium* is well-known important bacteria in early life for establishing gut microbiota, we examined an influence of early-life AMP on its amount in fecal samples. Significant differences among the experimental groups (NC, PC and AMP-treated) were not detected at 4, 8, or 28 weeks of age (Kruskal–Wallis test). Since fecal microbiota composition and relative abundance of *Bifidobacterium* at 4 weeks of age varied among AMP-treated groups I, II, and III (Fig 3c, Table S1), we further compared the amount of *Bifidobacterium* among the 5 groups (NC, PC, AMP-treated-I, II, and III) using non-parametric multiple comparisons (Fig. 4). Amount of *Bifidobacterium* in AMP-treated-III group was significantly lower compared to NC, PC and AMP-treated II group ($p<0.05$, Steel–Dwass test). Although AMP in early stage of life strongly perturbed the gut microbiota, amount of *Bifidobacterium* at 4 weeks of age varied among AMP-treated groups with at least partially dependent on difference of transferred bacteria from mother mouse during delivery and nursing.

Organic acid composition in cecal contents

To examine the impact of early-life AMP on the intestinal environment in western diet-fed mice, organic acid composition in cecum contents was analyzed at the end of the experiment

(Table 3). Major microbial metabolites, acetate, propionate, and butyrate were reduced in both PC group and AMP-treated groups, and concentration of acetate, butyrate, and sum of organic acids were significantly lower compared to NC group, probably attributable to western diet feeding. Although early-life AMP did not significantly affect the organic acid profiles, branched-chain organic acids (*iso*-valerate, *iso*-butyrate) were detected in some individuals, potentially reflecting the influence of western diet feeding and/or AMP treatment.

Association between gut microbiota and histological severity of steatotic liver disease

To explore relationships between the features of gut microbiota in the experimental groups and MASLD development, we tried to search explanatory variables to predict high or low NAS from 16S metagenomic data. In the random forest model, α-diversity at 4 weeks of age, relative abundance of *Firmicutes* at 28 weeks of age, *Actinobacteria* at 4 weeks of age, *Proteobacteria* at 4, 8, and 28 weeks of age, and *Deferribacteres* at 4 weeks of age were selected as candidates to predict the low NAS class, whereas α-diversity at 28 weeks of age, relative abundance of *Firmicutes* at 4 weeks of age were candidates to predict the high NAS class. For these attributes, correlations with severity of MASLD were assessed by Spearman's correlation coefficient by rank test. Among these, α-diversity (Shannon index) at 4 weeks of age was significantly lower in the low NAS (≤ 3) group compared to the others (4 ≤) ($p<0.05$, Mann–Whitney U-test) (Fig 5). Significant correlation was observed between Shannon index and combined NAS + fibrosis score ($rs = -0.3948$, $p<0.05$, Spearman's rank correlation), while Shannon index tended to correlate with NAS ($rs = -0.3747$, $p = 0.0515$). Amount of fecal *Bifidobacterium* at 4 weeks of age tended to be lower in the high NAS group, but the difference between high NAS group and the other were not statistically significant (Mann–Whitney U test). Correlation between *Bifidobacterium* abundance and NAS or NAS + fibrosis scores were also not significant. The other attributes of gut microbiota also did not significantly corelate with the MASLD severity on liver tissue. These findings suggest that early-life AMP-induced dysbiosis, particularly the reduction in microbial diversity at 4 weeks of age, might be important factor to increase susceptibility to steatohepatitis development.

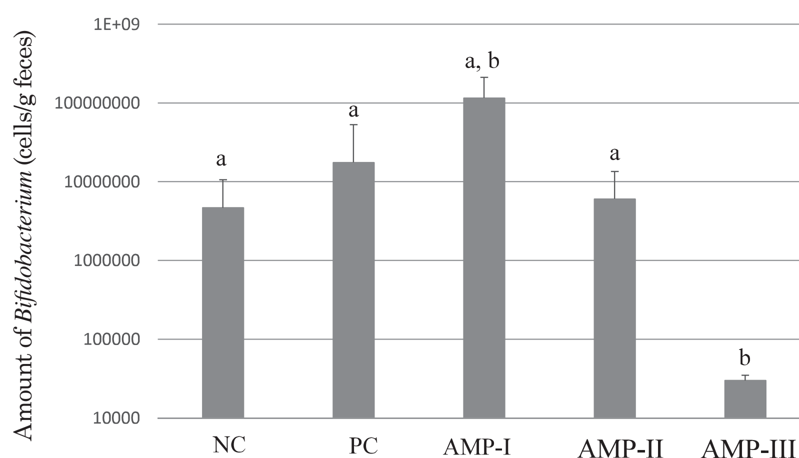

Figure 4. Effect of ampicillin exposure in early life on the amount of *Bifidobacterium* in mice feces at 4 weeks of age. The data are expressed as mean ± SD. Data with different letters are significantly different ($p<0.05$, Steel-Dwass test).

Table 3. Organic acids in cecum contents of mice at the end of experimental period.

	Organic acids concentration in cecum contents (μmole/g)									
	Succinic acid	Lactic acid	Formic acid	Acetic acid	Propionic acid	*iso*-Butyric acid	*n*-Butyric acid	*iso*-Valeric acid	*n*-Valeric acid	Sum
Negative control (n = 4)	0.97 ± 0.50	4.78 ± 1.76	ND	49.09 ± 10.44	7.59 ± 0.43	ND	17.19 ± 4.82	ND	ND	79.62 ± 16.27
Positive control (n = 4)	0.81 ± 0.20	5.72 ± 1.94	ND	21.69 ± 8.59**	3.04 ± 1.79	ND	3.38 ± 1.30*	0.98 (n = 1)	1.47 (n = 1)	35.24 ±14.29**
AMP-treated (n = 14)	1.09 ± 0.58	4.35 ± 1.30	ND	22.41 ± 6.24**	2.14 ± 1.14	2.04 (n = 1)	3.99 ± 1.37*	1.71 ± 0.76 (n = 4)	ND	34.46 ± 9.59**

Values are mean ± SD.
Some samples in PC and AMP-treated group were pooled before analysis because of their too small volume.
Results from male and female samples are combined and statistically compared.
Significant difference from negative control (*$p<0.05$; **$p<0.01$, Welch's t-test).

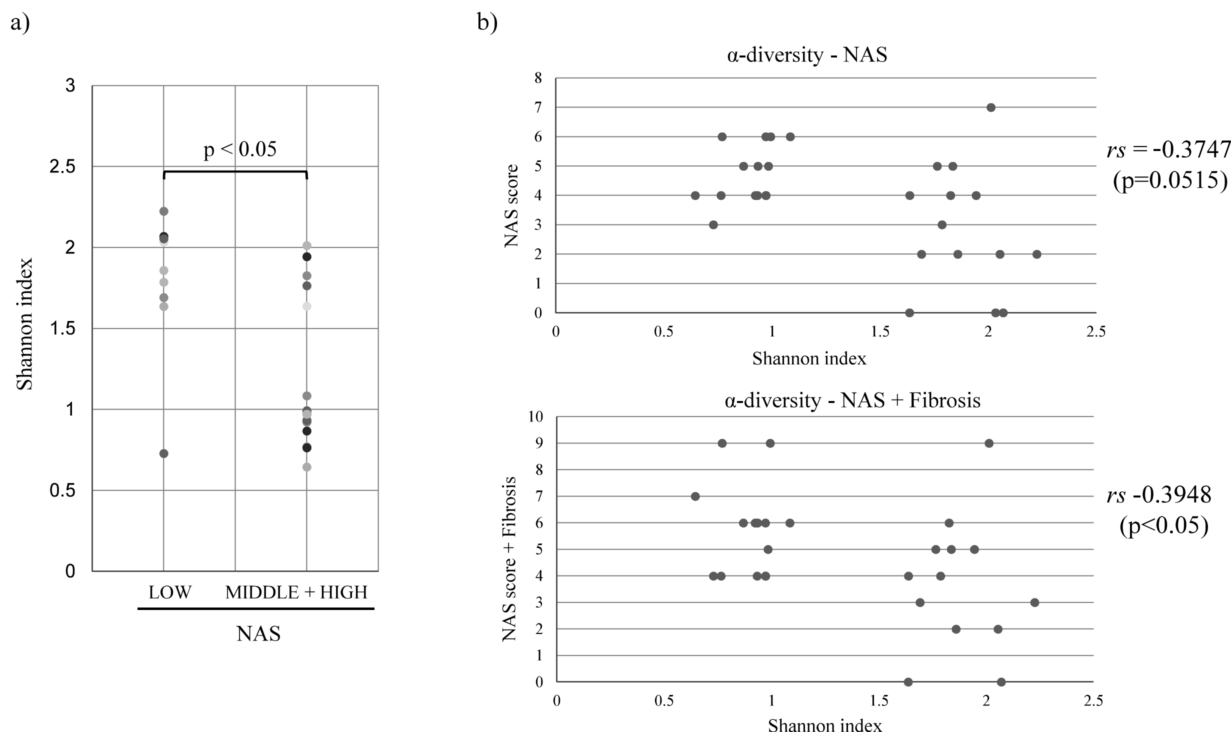

Figure 5. Negative correlation between MASLD activity and α-diversity of fecal microbiota at 4 weeks of age. a) α-Diversity (Shannon index) was compared between Low NAS group and Middle + High NAS group as described in Material and Methods. Significant differences were examined by Mann-Whitney U-test. b) Correlation analysis between the α-diversity of microbiota and histological severity of MASLD by Spearman's signed rank test.

DISCUSSION

In this study, we examined effects of early-life AMP from birth to weaning on the onset and severity of steatotic liver disease in western diet-fed adult mice. We also examined the effects of AMP treatment on fecal microbiota composition at 4, 8, 28 weeks of age and on organic acids composition in cecum contents at the end of experiment, and then association of antibiotic-induced changes of gut environment with the severity of MASLD was examined.

Western diet used in this study induced hepatic steatosis and some male mice in PC group developed MASH with elevated serum ALT as described by DeLeve *et al.* (27). On the other hand, almost female mice in the PC group had a lower level of hepatic steatosis and no mice were judged with MASH. In AMP-treated group female mice tend to be high in grades of steatosis, lobular inflammation and NAS. Three of AMP-treated mice showed more severe liver tissue fibrosis accompanied by gross liver atrophy and elevated serum TBA levels at the end of the experiment. Compared with PC group, lobular inflammation grade was significantly high in AMP-treated group. and the occurrence of more severe MASH in some AMP-exposed mice with dependent on the difference of dam. These results suggest that early-life AMP may predispose to the disease through interfering with gut microbiota developing process.

Individually different developments of MASH have been reported to be at least partially dependent on the compositional differences and susceptibility to dysbiosis in the gut microbiota in both humans and experimental animal models (1, 11, 13, 18-21). Le Roy *et al.* (18) have reported that donation of intestinal microbiota from high fat diet-responder mice promoted more development of steatohepatitis in the receiver mice. Generally, gut microbiota begins to be formed just after birth and the development is thought to be influenced by many factors such as maternal microorganisms, breast milk components, genetic background, host-microbe interactions, and microbe-microbe interactions (1, 3-5), resulting in the establishment of a individually-specific gut microbiota until 3-4 weeks of age in mice (7) and around 3 years of age in humans (3, 6). The early stage of life has been recognized as a critical period for establishing stable microbiota and maintaining resilience of microbiota throughout life (3-5, 9). Among various kinds of environmental factors, antibiotic use, particularly during infancy, could deeply modify intestinal microbiota and intestinal environment and impact on short- and long-term health (23, 25, 26).

So, we performed 16S metagenomic analysis of fecal microbiota at 4, 8 and 28 weeks of age to examine the effects of ampicillin exposure on fecal microbiota and tried to clarify important features of the microbiota correlating with the severity of MASH. At 4 weeks of age, just after exposure to 1g/L ampicillin for 3 weeks from birth, greatly reduced the diversity of fecal microbiota and changed the microbiota composition to be markedly biased (Fig. 3, Table S1). At 8 weeks of age, compositional difference between AMP-treated and untreated mice became smaller than at 4 weeks of age, but significant decrease of α-diversity remained. Furthermore, the bacterial composition at 28 weeks of age after feeding a western diet also differed between the AMP-exposed and PC groups (Fig. 3c). The concentration of ampicillin was to induce a seemingly germfree state as described in Castro-Mejia *et al.* (28), so that AMP treatment in our study strongly impact on fecal microbiota and the influences persisted over a long period. These results indicate a long-term continuing effect of early -life

AMP on fecal microbiota. The western diet also affected the composition of the fecal microbiota in mice at 28 weeks of age (Fig. 3, Table S1). Compared with the NC group, the PC group had a different microbiota composition, specifically lowered proportions of *Bifidobacterium, Lactobacillus, Turicibacter, Christecenenella* and *Bacteridales* S24-7 as previously reported in diet-induced MASH model mice (12, 34, 35). Microbiota composition in the AMP-exposed group was also affected by the western diet but differed from that in the PC group. *Parabacteroides*, which were highly prevalent in the PC group, were significantly lower in the AMP-treated group. *Turicibacter* and *Christencenellaceae* were rather minor population in PC group and became moreover decreased in the AMP-treated group. These bacterial species which were lowered in AMP-treated group have been reported to be negatively correlated with obesity and obesity-related metabolic disorders, through producing organic acids, modifying bile acid metabolism, strengthening the intestinal barrier, or exerting anti-inflammatory effects (36-41).

Several studies have reported the effects of early-life antibiotic exposure on metabolic disorders and steatotic liver disease. Cox LM *et al*. (26) showed that low-dose penicillin (6.67 mg/L as drinking water) perturbed the microbiota transiently but was sufficient to induce lasting metabolic consequences. Nobel YR *et al*. (42) used therapeutic-dose pulsed antibiotic treatment (PAT, 0.167mg/ml Amoxicillin and/or 0.333mg/ml Tylosin as drinking water) and showed that early-life PAT accelerated total body mass and bone growth. They further demonstrated PAT-mediated progressive changes in gut microbiome and its influence on microbiota adaptation to a change of diet from normal chow to high fat diet. In our study, early-life AMP was performed at higher concentration as described in Castro-Mejia *et al*. (28) than in the above reports (26, 42), and its influence was observed even after western diet feeding. Miao ZH *et al*. (43) investigated effects of higher dose of ceftriaxone (CTRX, 100 mg/kg once daily from 2 to 4 weeks of age) on the gut microbiota composition and the association between high fat diet-induced dysmetabolism and dysbiosis of the gut microbiota. They showed that CTRX exposure significantly decreased α-diversity just after exposure, and the difference of microbiota composition between CTRX-treated and nontreated group continued to the end of their experiment. Considering these previous studies and the above results of our study, antibiotic exposure-mediated disturbance of gut microbiota in early life until completing the weaning is thought to be one of the important factors affecting the severity of diet-induced steatotic liver disease in adulthood. Bacterial metabolites such as organic acids and secondary bile acids have also been known to have suppressing effects on obesity-related onset and progression of MASH (1, 11, 13, 15, 16). Then, we analyzed organic acid composition in the cecal contents at the end of experimental period. Long-term feeding of the western diet significantly decreased main organic acids, but no significant effect of AMP exposure was detected (Table 3).

To identify the features of fecal microbiota as explanatory variables that could predict the Low or High NAS group, we utilized random forest classification. Among the identified features of microbiota, only the lower α-diversity (Shannon index) of fecal microbiota at 4 weeks of age significantly correlated with more severe steatohepatitis. These results indicate that reduced diversity of fecal microbiota at the age of 4 weeks of age in the AMP-treated group is an important factor associated with severity of steatotic liver disease. Ampicillin-mediated decrease of multiple bacterial species, which could adapt to western diet and involve in metabolic homeostasis, may potentially promote the development to more advanced MASH.

Sequential colonization of a diverse range of gut bacteria during early life are essential to establish and maintain a stable symbiotic relationship between gut microbiota and the host throughout life (3, 44). During this "neonatal window of opportunity "(44), a transient immune response (weaning reaction) occurs against the increased number of *Clostridia* and other microorganisms. Meanwhile, gut-specific regulatory T cells are induced through interaction with gut microbiota-derived bacterial antigens and bacterial metabolites, especially butyrate to provide a protective role against the weaning reaction (45-47). The immune system imprinting by gut microbiota has an important role in long-term immune tolerance to the intestinal microbiota, which may contribute to future susceptibility to immune and inflammatory diseases. In addition to promotion of well-balanced T cell development, secretory IgA has been known to contribute to forming and maintaining the intestinal environment (48). Supporting role of some gut bacteria SFB (49), *Limosilactobacillus* and *Muribaculaceae* (50) in induction of IgA production have been reported. In AMP-treated mice of present study, significantly reduced α-diversity and decrease of relative abundance of *Clostridium* and *Muribaculaceae* (the family name given to *Bacteroidales* S24-7) were observed at 4 weeks of age, and lobular inflammation in the liver tissue was significantly higher compared to NC group. This dysbiosis of the gut environment in early life may probably affect the risk of developing steatohepatitis in western diet-fed adult mice, although further detailed analysis of constitutive species of gut microbiota and the composition of their metabolite SCFAs at this early stage of life, as well as bacterial load are required.

Bifidobacterium transferred from mother during delivery and nursing is one of the important constituents of the juvenile gut microbiota to establish resilient gut environment (1-5). It has been reported to promote the colonization of other intestinal bacteria and affect their gene expression (51, 52). The intake of *Bifidobacteria* as a probiotic could stabilize the balance of healthy intestinal microbiota (53, 54). In this study, relative abundance of phylum *Actinobacteria* at 4 weeks of age was selected as a potential predictor for high NAS group. Although significant correlation with the severity of steatohepatitis was not found, AMP-treated group- III had extremely low *Bifidobacterium* loads (Fig. 4) and some mice in this group developed advanced MASH with higher stage of fibrosis (Table 2), suggesting the possibility of protective role by *Bifidobacterium* in early life against western diet-induced MASH. Presence of naturally antibiotic-insensitive *Bifidobacteria* (55) and their role in keeping resilience of gut microbiota (56) have been recently reported. It is possible that ampicillin insensitive or low-sensitive *Bifidobacterium* derived from some mother mice may happen to be transferred and be kept in offspring under AMP treatment.

Our study has following limitations. Due to the small number of mice in experimental groups, sex-dependent difference could not be statistically analyzed. Gender differences in the gut microbiota exist in both model animals and humans, and their association with the onset of various diseases including metabolic syndrome, has been attracting attention (57-59). It is important to examine the association of antibiotic exposure-mediated temporal changes in the gut microbiota with the progression of steatotic liver disease in both sexes separately. Secondary, composition of bacterial metabolites at earlier age should be analyzed. Total bacterial load in fecal microbiota should also be counted. Finally, fecal microbiota in maternal mice should be analyzed to consider what kind of differences of the gut microbiota derived from mother mice influenced the infant's gut microbiota and the diet-induced steatotic liver disease.

In this study, we examined effects of AMP in early life on the temporal changes of fecal microbiota and severity of MASLD in western diet-fed C57BL/6J mice. Histological evaluation of MASLD showed that steatosis in female mice and lobular

inflammation was significantly influenced with AMP, and that NAS tended to be high in female of AMP-treated group. 16S metagenome analyses of fecal microbiota showed significant decrease of α-diversity and remarkable shift to normally minor bacterial species at 4 weeks of age in AMP-treated mice, and the influence was continuously observed even after finishing the western diet feeding period. Organic acid composition in cecal contents was decreased in western diet-fed group, but no influence with AMP treatment. α-Diversity at 4weeks of age negatively correlated with combined scores of steatohepatitis and fibrosis. These results suggest that AMP in early life induced dysbiosis of gut microbiota and could promote the development of western diet-associated steatotic liver disease.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest for this article.

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AUTHOR CONTRIBUTIONS

Conceptualization ; R.I., K.K. and A.S., Methodology ; R.I., M.N., A.S., H.N-I., T.K., M.I-S., and M.S. ; Writing-original draft preparation ; R.I. ; Writing-review and editing ; R.I., K.K., M.I-S., T.K., M.S., Supervision ; R.I., K.K.

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Supplemental Table 1. Relative abundance of 65 main genera in individual fecal samples at 4, 8, and 28 weeks of age.

< 65 main genera from fecal samples >

	Kingdom	Phylum	Class	Order	Family	Genus	ID
OTU1	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Curtobacterium	Curtobacterium
OTU2	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Frigoribacterium	Frigoribacterium
OTU3	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Leucobacter	Leucobacter
OTU4	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Pseudoclavibacter	Pseudoclavibacter
OTU5	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	Rhodococcus
OTU6	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Sanguibacteraceae	Sanguibacter	Sanguibacter
OTU7	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	Bifidobacterium
OTU8	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Coriobacteriaceae_UKG	{Unknown Genus} Coriobacteriaceae
OTU9	Bacteria	Actinobacteria	Coriobacteriia	Coriobacteriales	Coriobacteriaceae	Adlercreutzia	Adlercreutzia
OTU10	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidales_UKF	Bacteroidales_UKG	{Unknown Family} Bacteroidales
OTU11	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	Bacteroides
OTU12	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Porphyromonadaceae	Parabacteroides	Parabacteroides
OTU13	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Rikenellaceae	Rikenellaceae_UKG	{Unknown Genus} Rikenellaceae
OTU14	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	S24-7_UKF	S24-7_UKG	{Unknown Genus} S24-7
OTU15	Bacteria	Deferribacteres	Deferribacteres	Deferribacterales	Deferribacteraceae	Mucispirillum	Mucispirillum
OTU16	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planococcaceae_UKG	{Unknown Genus} Planococcaceae
OTU17	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Sporosarcina	Sporosarcina
OTU18	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Jeotgalicoccus	Jeotgalicoccus
OTU19	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	Staphylococcus
OTU20	Bacteria	Firmicutes	Bacilli	Lactobacillales	Carnobacteriaceae	Granulicatella	Granulicatella
OTU21	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcaceae_UKG	{Unknown Genus} Enterococcaceae
OTU22	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Enterococcus
OTU23	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus	Vagococcus
OTU24	Bacteria	Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	Lactobacillus
OTU25	Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Leuconostoc	Leuconostoc
OTU26	Bacteria	Firmicutes	Bacilli	Lactobacillales	Leuconostocaceae	Weissella	Weissella
OTU27	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	Lactococcus
OTU28	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus
OTU29	Bacteria	Firmicutes	Bacilli	Turicibacterales	Turicibacteraceae	Turicibacter	Turicibacter
OTU30	Bacteria	Firmicutes	Clostridia	Clostridia_UKO	Clostridia_UKF	Clostridia_UKG	{Unknown Order} Clostridia
OTU31	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiales_UKF	Clostridiales_UKG	{Unknown Family} Clostridiales
OTU32	Bacteria	Firmicutes	Clostridia	Clostridiales	[Mogibacteriaceae]	[Mogibacteriaceae]_UKG	{Unknown Genus} [Mogibacteriaceae]
OTU33	Bacteria	Firmicutes	Clostridia	Clostridiales	Christensenellaceae	Christensenellaceae_UKG	{Unknown Genus} Christensenellaceae
OTU34	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridiaceae_UKG	{Unknown Genus} Clostridiaceae
OTU35	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	02d06	02d06
OTU36	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Candidatus Arthromitus	Candidatus Arthromitus
OTU37	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	Clostridium
OTU38	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	SMB53	SMB53
OTU39	Bacteria	Firmicutes	Clostridia	Clostridiales	Dehalobacteriaceae	Dehalobacterium	Dehalobacterium
OTU40	Bacteria	Firmicutes	Clostridia	Clostridiales	Eubacteriaceae	Anaerofustis	Anaerofustis
OTU41	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Lachnospiraceae_UKG	{Unknown Genus} Lachnospiraceae
OTU42	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	[Ruminococcus]	[Ruminococcus]
OTU43	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Coprococcus	Coprococcus
OTU44	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Dorea	Dorea
OTU45	Bacteria	Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Roseburia	Roseburia
OTU46	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Peptococcaceae_UKG	{Unknown Genus} Peptococcaceae
OTU47	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptococcaceae	Rc4-4	Rc4-4
OTU48	Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	Peptostreptococcaceae_UKG	{Unknown Genus} Peptostreptococcaceae
OTU49	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcaceae_UKG	{Unknown Genus} Ruminococcaceae
OTU50	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Oscillospira	Oscillospira
OTU51	Bacteria	Firmicutes	Clostridia	Clostridiales	Ruminococcaceae	Ruminococcus	Ruminococcus
OTU52	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Erysipelotrichaceae_UKG	{Unknown Genus} Erysipelotrichaceae
OTU53	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	Allobaculum
OTU54	Bacteria	Firmicutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Coprobacillus	Coprobacillus
OTU55	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	Methylobacterium
OTU56	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonadaceae_UKG	{Unknown Genus} Sphingomonadaceae
OTU57	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas
OTU58	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Sutterella	Sutterella
OTU59	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Curvibacter	Curvibacter
OTU60	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	Enterobacteriaceae_UKG	{Unknown Genus} Enterobacteriaceae
OTU61	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxellaceae_UKG	{Unknown Genus} Moraxellaceae
OTU62	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas
OTU63	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Stenotrophomonas	Stenotrophomonas
OTU64	Bacteria	Tenericutes	Mollicutes	RF39	RF39_UKF	RF39_UKG	{Unknown Family} RF39
OTU65	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Akkermansia	Akkermansia

	< 4 weeks of age, Male >	Relative abundance (%)															
		Negative control (NC)		Positive control (PC)			AMP-treated - I	AMP-treated - II				AMP-treated - III					
	ID	Male - 1	Male - 2	Male - 1	Male - 2	Male - 3	Male - 1	Male - 1	Male - 3	Male - 4	Male - 6	Male - 1	Male - 2	Male - 3	Male - 4	Male - 5	Male - 6
OTU1	Curtobacterium	0	0	0	0	0	0.000765285	0	0	0	0	0	0	0	0	0.002601964	0
OTU2	Frigoribacterium	0	0	0	0	0	0.00051019	0	0	0	0.002643981	0	0.002391801	0	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0.002550949	0	0	0	0	0	0.003587701	0	0.003797613	0	0
OTU4	Pseudoclavibacter	0	0	0	0.001458045	0	0.002040759	0	0	0.002522323	0.002643981	0.0012782	0.002391801	0	0.002531742	0	0
OTU5	Rhodococcus	0	0	0.002845598	0	0	0.000765285	0	0	0	0.001321991	0	0	0	0	0	0
OTU6	Sanguibacter	0	0	0	0	0	0.001530569	0	0	0	0.001982986	0	0.005979502	0	0	0	0
OTU7	Bifidobacterium	0.22136954	0.117468995	0.042683968	0.163301013	0.116029628	27.95023609	12.34257826	0.007805183	0.617969026	0.048913654	0	0.002391801	0.002555649	0.001265871	0.002601964	0
OTU8	{Unknown Genus} Coriobacteriaceae	0.068870523	0.083583708	0.002845598	0.018954582	0.035078725	0	0	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.91991342	0.815505907	0.320129759	0.405336444	0.465467694	0	0	0	0.005044645	0	0	0	0	0	0	0
OTU10	{Unknown Family} Bacteroidales	0.014757969	0.009036077	0.001422799	0.00291609	0	0	0	0	0	0	0	0	0	0	0	0
OTU11	Bacteroides	0.20661157	0.526351458	0.367082124	0.075818328	0.573402232	0.001530569	0	0	0	0	0	0	0	0	0	0
OTU12	Parabacteroides	0.319756002	0.894571577	0.004268397	0.037909164	0.450626695	0	0	0	0.002522323	0.000660995	0	0	0	0	0	0
OTU13	{Unknown Genus} Rikenellaceae	6.109799292	3.815483317	8.438620454	4.344973391	3.440413389	0.002550949	0.000713526	0	0.002522323	0	0.0012782	0	0.003833474	0	0	0.005143239
OTU14	{Unknown Genus} S24-7	24.680244	28.35069013	24.76096978	20.32222789	27.77155655	0.006122277	0.008562316	0.000867243	0.005044645	0.001982986	0.0012782	0.009567204	0.010222597	0	0	0.003857429
OTU15	Mucispirillum	0.191853601	0.160390359	0.179272665	0.246409565	0.275233071	0.001530569	0.001427053	0	0	0	0	0	0	0	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0	0.001530569	0	0	0	0	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0	0	0	0.00051019	0	0	0	0	0	0	0	0	0	0
OTU18	Jeotgalicoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU19	Staphylococcus	0	0.002259019	0	0.00291609	0	9.594118532	0	0.000867243	0	0.000660995	0	0.003587701	0	0.003797613	0.001300982	0.00128581
OTU20	Granulicatella	0.034435262	0.004518038	0.011382391	0.005832179	0.002698363	0.00051019	7.304368922	0.042494883	0.017656258	0.048252659	13.52463731	17.43264091	10.17531754	9.019329848	16.84121512	13.78388109
OTU21	{Unknown Genus} Enterococcaceae	0	0	0	0	0.006745909	0	2.590814062	0	0	0	0	0	0.002555649	0	0.003902947	0
OTU22	Enterococcus	0.049193231	0.056475478	0.055489158	0.023328716	0.012142635	0.002806044	25.32162199	36.68956187	47.11698532	70.56455611	52.66440851	68.66740812	45.67200797	38.32170842	63.07161907	55.16124055
OTU23	Vagococcus	0	0	0	0	0	0	0.000713526	0.001734485	0.017656258	0.015863888	0.007669202	0.010763104	0.005111299	0.008861096	0.007805893	0.007714859
OTU24	Lactobacillus	25.5952381	30.70007003	35.904331	40.2216228	20.57906879	0.745387247	24.53888362	50.2142089	31.357514	0.522186308	0.002556401	0.020330308	0.031945616	0.011392838	0.01170884	0.006429049
OTU25	Leuconostoc	0	0	0	0	0.002698363	0	0	0	0	0	0	0	0	0	0	0
OTU26	Weissella	0	0	0	0	0	0.000255095	0	0	0	0	0	0	0	0	0	0
OTU27	Lactococcus	0	0	0	0.001458045	0	0.00051019	0	0	0.002522323	0	0	0	0	0	0	0.00128581
OTU28	Streptococcus	0	0	0.002845598	0.001458045	0.002698363	0.006377372	0	0	0	0.002643981	0.0012782	0.0011959	0.001277825	0.002531742	0	0
OTU29	Turicibacter	3.473042109	7.188198884	0.004268397	0	0.004047545	61.62454625	19.95376349	13.00083255	20.80916108	28.76651662	33.75471336	13.63087337	43.94438907	52.58300948	19.95706759	31.01630407
OTU30	{Unknown Order} Clostridia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU31	{Unknown Family} Clostridiales	21.44824872	12.54207423	16.27539696	18.20806299	27.37489712	0.029591006	0.004994684	0.002601728	0.007566968	0.003304977	0.0012782	0.003587701	0.015333896	0.001265871	0.002601964	0.003857429
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.083628493	0.02484921	0.019919185	0.048115477	0.044522997	0	0	0	0	0	0	0	0	0	0	0
OTU33	{Unknown Genus} Christensenellaceae	0.059031877	0.05195744	0.017073587	0.018954582	0.020237726	0	0	0	0	0	0	0	0	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.167256985	0.146836244	0.081099539	0.15601079	0.087696812	0	0	0	0	0	0	0	0	0	0	0
OTU35	02d06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU36	Candidatus Arthromitus	1.47579693	2.58205887	2.919583404	1.704454327	1.923933135	0.002295854	0	0	0.002522323	0	0	0	0.001277825	0	0	0
OTU37	Clostridium	0	0	0.234761823	0.068528104	1.129265101	0	0	0	0	0	0	0	0	0	0	0
OTU38	SMB53	0	0.049698421	0	0	0	0	0	0	0	0	0	0	0	0	0.001300982	0
OTU39	Dehalobacterium	0.029515939	0.02710823	0.071139947	0.072902238	0.124124718	0.000255095	0	0	0	0	0	0	0.001277825	0	0	0
OTU40	Anaerofustis	0.093467139	0.045180383	0.018496386	0.03061894	0.035078725	0	0	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	4.74222747	3.018049563	3.437482215	4.8990304	3.83302527	0.004846803	0.002140579	0	0.002522323	0	0	0	0.005111299	0	0	0
OTU42	[Ruminococcus]	2.499016135	1.260532677	0.486597234	1.113946198	0.623321955	0	0	0	0.002522323	0	0	0	0.002555649	0	0	0
OTU43	Coprococcus	0.14266037	0.151354282	0.210574242	0.291608952	0.596338321	0	0.000713526	0	0	0	0.0012782	0	0	0	0	0
OTU44	Dorea	0.024596616	0.106173899	0.122360708	0.145804476	0.275233071	0.000255095	0	0	0	0	0	0	0	0	0	0
OTU45	Roseburia	0.20661157	0.05195744	0.143702692	0.265364147	0.022936089	0.00051019	0	0	0	0	0.0012782	0	0	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0.007113995	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.944510035	1.416404997	0.735587047	0.491361085	0.720463039	0	0	0.000867243	0	0	0	0	0.001277825	0	0	0.00128581
OTU48	{Unknown Genus} Peptostreptococcaceae	0	0.038403325	0	0	0	0	0	0	0.002522323	0	0	0	0	0	0	0
OTU49	{Unknown Genus} Ruminococcaceae	3.330381739	2.900580568	2.359000626	2.913173434	4.054291072	0.003571328	0.001427053	0.002601728	0.007566968	0	0	0	0.002555649	0	0	0.00128581
OTU50	Oscillospira	1.569264069	0.987191362	1.466905697	2.101042502	2.699712624	0.00102038	0	0.000867243	0	0	0	0	0.006389123	0.001265871	0	0
OTU51	Ruminococcus	0.978945297	0.557977726	0.774002618	0.959393453	1.12117001	0.00102038	0	0	0.005044645	0	0.0012782	0	0	0	0	0
OTU52	{Unknown Genus} Erysipelotrichaceae	0.147579693	0.869722367	0.206305845	0.128307939	0.237455983	0	0	0	0	0	0	0	0	0	0	0
OTU53	Allobaculum	0	0	0	0	0	0	0.000713526	0.000867243	0.002522323	0	0	0	0	0	0	0
OTU54	Coprobacillus	0	0	0.007113995	0.017496537	0.410151243	0	0	0	0	0	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0.003826423	0.000713526	0	0	0	0	0	0	0.001265871	0	0.00257162
OTU56	{Unknown Genus} Sphingomonadaceae	0	0	0	0	0	0.002040759	0.019978737	0.013008638	0.005044645	0.005287963	0.014060203	0.112414643	0.062613407	0.016456321	0.048136343	0.00128581
OTU57	Sphingomonas	0	0	0	0	0	0.00051019	0.003567632	0	0	0.001982986	0.006391001	0.021526208	0.007666948	0.005063483	0.009106876	0.00128581
OTU58	Sutterella	0.059031877	0.126505071	0.049797963	0.148720566	0.143013262	0	0	0	0	0	0	0	0	0	0	0
OTU59	Curvibacter	0	0	0	0	0	0.000765285	0.005708211	0.001734485	0	0.000660995	0	0.010763104	0.005111299	0	0.005203929	0
OTU60	{Unknown Genus} Enterobacteriaceae	0.019677292	0.065511555	0.149393888	0.010206313	0.118727991	0	7.885179345	0	0	0.000660995	0	0.002391801	0.001277825	0	0.002601964	0.00128581
OTU61	{Unknown Genus} Moraxellaceae	0	0.009036077	0	0	0	0.002295854	0.011416421	0.019079335	0.005044645	0.007270949	0.008947402	0.05381552	0.037056914	0.01519045	0.031223574	0
OTU62	Pseudomonas	0	0	0	0	0	0.00051019	0	0	0	0	0.006391001	0.0011959	0.001277825	0	0	0
OTU63	Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU64	{Unknown Family} RF39	0.029515939	0.02484921	0.032724375	0.007290224	0.012142635	0	0	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.0639512	0.221383875	0.075408343	0.323685937	0.654353135	0	0	0	0	0	0	0.0011959	0	0.001265871	0	0
	Bacteroidetes/Firmicutes	0.467	0.519	0.512	0.335	0.489	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	< 8 weeks of age, Male >	Relative abundance (%)															
		Negative control (NC)		Positive control (PC)			AMP-treated - I	AMP-treated - II				AMP-treated - III					
	ID	Male - 1	Male - 2	Male - 1	Male - 2	Male - 3	Male - 1	Male - 1	Male - 3	Male - 4	Male - 6	Male - 1	Male - 2	Male - 3	Male - 4	Male - 5	Male - 6
OTU1	Curtobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU2	Frigoribacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU4	Pseudoclavibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU5	Rhodococcus	0	0	0	0	0	0	0	0	0.002616123	0.002582311	0	0	0	0	0	0
OTU6	Sanguibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU7	Bifidobacterium	0	0	2.468906892	2.70160238	2.078079847	0.094172515	0.906022994	0.079092118	0.846315845	0.24273725	0.325465927	0.18639329	0.608432007	0.385392974	0.429686898	1.382819053
OTU8	{Unknown Genus} Coriobacteriaceae	1.117581257	1.073543457	1.103947969	0.574142701	0.612012001	0	0	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.347087912	0.407513531	0.482085806	0.307949267	0.239282888	0.497769008	0.20362638	0.36190636	0.219754346	0.205293738	0.463254422	0.261213132	0.288851559	0.299469295	0.27875745	0.390676732
OTU10	{Unknown Family} Bacteroidales	0	0.00254696	0	0	0	0	0.001143968	0	0	0.001291156	0	0	0	0	0	0
OTU11	Bacteroides	1.379988082	0.854504935	0.229632588	0.630513075	0.408621547	0	0	0	0	0	0	0	0.135207113	0	0	0
OTU12	Parabacteroides	1.286943431	0.808659663	0.78731173	1.531395167	1.513004105	0	0	0	0	0	0	0	0.059409186	0	0	0
OTU13	{Unknown Genus} Rikenellaceae	5.128955704	2.70614454	1.621691009	3.035649042	2.081761122	10.47893451	1.699937082	1.698083814	3.601093539	4.04389929	4.95563448	11.84910019	5.920432663	8.759160981	4.495541421	5.126252593
OTU14	{Unknown Genus} S24-7	22.26589861	18.12543776	16.8316408	19.95511248	16.97620056	24.01062804	28.79597323	19.20500437	20.21216759	18.23499032	26.62643876	17.61285326	13.64362683	26.66919383	11.91110564	22.39328124
OTU15	Mucispirillum	0.313633655	1.739573384	1.88840712	1.33827444	2.396510151	0	0	0	0	0	0	0	0	0	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU18	Jeotgalicoccus	0	0	0	0	0	0.013453216	0	0	0	0	0	0	0	0	0	0
OTU19	Staphylococcus	0.004181782	0.00127348	0	0.001043896	0.001840638	0.003363304	0	0	0	0	0.002375664	0	0.002048593	0.002527167	0	0
OTU20	Granulicatella	0	0.00127348	0	0.003131687	0	0.008968811	0.001143968	0	0.001308062	0	0.002375664	0	0.00819437	0.001263584	0.004620289	0.002207213
OTU21	{Unknown Genus} Enterococcaceae	0	0	0.001426289	0	0	0.013453216	0.001143968	0	0.005232246	0	0.004751327	0.005250515	0.010242963	0.005054334	0.003080193	0
OTU22	Enterococcus	0.004181782	0	0.004278868	0.002087792	0.001840638	0.016816521	0.004575874	0.003595096	0.00784837	0.001291156	0.01306615	0	0.022534519	0.012635835	0.001540096	0.011036066
OTU23	Vagococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU24	Lactobacillus	4.457779683	10.40432983	9.320801004	10.74586356	12.29914042	17.31877396	49.12886804	11.96807554	20.23440464	12.15235636	23.92174564	11.89766746	24.56877125	23.09451605	14.91583373	24.22195736
OTU25	Leuconostoc	0.002090891	0.00127348	0.002852579	0	0	0.004484405	0	0	0	0	0	0.005250515	0	0.002527167	0.003080193	0
OTU26	Weissella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU27	Lactococcus	0.002090891	0	0	0	0	0	0	0	0	0	0.002375664	0	0	0.002527167	0	0.002207213
OTU28	Streptococcus	0.004181782	0.00127348	0	0	0	0	0.001143968	0	0.002616123	0.001291156	0.002375664	0	0.002048593	0.011372252	0.001540096	0
OTU29	Turicibacter	0.001045446	0.003820439	1.072569603	0.489587139	1.328940345	9.468822171	0.55253675	0.666291179	1.105312038	0.417043254	0.009502655	0.018376803	0.190519113	0.039171089	0.063143953	0.065112789
OTU30	{Unknown Order} Clostridia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU31	{Unknown Family} Clostridiales	40.96787346	37.74212034	37.99064354	30.33561251	32.91704246	19.03742236	9.674540983	39.55324937	35.4864027	40.1626856	28.22526043	38.66216873	37.47695333	26.70331059	46.61717824	35.70167307
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.076317523	0.109519261	0.13549749	0.061589853	0.081908373	0	0.008007779	0.031157501	0.018312862	0.027114267	0	0	0	0	0	0
OTU33	{Unknown Genus} Christensenellaceae	0.036590593	0.031836995	0.019968051	0.019834021	0.019326695	0	0	0	0	0	0	0	0	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.240452469	0.212671124	0.131218622	0.087687249	0.081908373	0.284759748	0.006863811	0	0	0	0	0	0	0	0	0
OTU35	02d06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU36	Candidatus Arthromitus	0	0.011461318	0.011410315	0.011482854	0.000920319	0	0	0	0	0	0.33378075	0.276964677	0.192567706	0.11119535	0.064684049	0.17768066
OTU37	Clostridium	0.013590792	0.019102197	0.671782291	0.297510308	0.131605588	0.468620373	0.032031116	0.004793462	0.020928985	0.037443512	2.337653082	0.37278658	0.473224894	0.476370988	0.215613497	1.362954134
OTU38	SMB53	0.017772574	0.006367399	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU39	Dehalobacterium	0.252997815	0.416427889	0.278126426	0.290203038	0.236521931	0	0	0	0	0	0	0	0	0	0	0
OTU40	Anaerofustis	0.016727128	0.033110474	0.057051575	0.017746229	0.016565738	0	0	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	2.713976561	3.791149316	7.9130534	10.23331072	10.73091719	7.277068993	3.180232226	10.74574281	5.85619171	9.575209813	1.986054854	6.595959729	4.631867907	3.35355067	5.644453343	2.458835474
OTU42	[Ruminococcus]	2.504887458	2.782553327	1.606001826	1.349757294	1.197334757	2.034798987	0.633758508	1.876640263	1.364308232	1.864428664	1.276919239	2.327290865	2.085467284	1.741218095	2.830697202	0.750452479
OTU43	Coprococcus	0.085726532	0.248328558	0.677487449	1.38838144	1.155920411	0.863248055	0.264256707	0.72620945	0.623945375	0.770819884	0.327841591	0.748198417	0.811242676	0.724033359	0.843972833	0.304595418
OTU44	Dorea	0.219543558	0.173193251	0.266716111	0.181637873	0.182223122	0.058297271	0	0	0	0	0.058203761	0.143076539	0.13315852	0.104877432	0.19713234	0.040833444
OTU45	Roseburia	0.001045446	0.017828717	0.049920128	0.302729788	0.024848608	0	0	0	0	0	0	0	0.00819437	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0.048493838	0.036536354	0.031290839	0	0	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.800811266	0.575612862	0.228206298	0.36953912	0.347880506	0.822888406	0.700108677	0.297194627	0.384570106	0.194964493	1.085678311	0.594620847	0.61867497	0.761940864	0.642220203	0.877367236
OTU48	{Unknown Genus} Peptostreptococcaceae	0.016727128	0.00254696	0	0	0.000920319	0	0	0	0.001308062	0	0	0	0	0	0	0
OTU49	{Unknown Genus} Ruminococcaceae	3.550332974	5.917860554	5.354290278	4.242392609	4.617239412	2.598712976	2.112909684	5.53764665	4.885610015	6.158812137	2.786653521	3.536221992	3.160978408	2.747030579	4.547904699	1.27687282
OTU50	Oscillospira	3.512696936	4.770455269	4.450022821	4.255963255	3.939884776	3.265768291	1.222902248	5.200905964	3.267537836	3.563589413	3.514794446	2.889095993	3.169172778	2.825372757	3.824059386	2.312055798
OTU51	Ruminococcus	6.656351604	5.361349889	2.276357827	2.867581815	2.514310957	1.107648154	0.463307213	1.695687083	1.522583683	2.108457069	1.634456626	1.936127483	1.577416315	1.120798585	2.411790978	1.105813799
OTU52	{Unknown Genus} Erysipelotrichaceae	0.965991657	0.716969118	0.345162026	0.194164622	0.090191242	0.230946882	0.395813076	0.345129244	0.285157425	0.228534538	0.092650884	0.040691493	0.036874667	0.029062421	0.043122699	0.027590165
OTU53	Allobaculum	0.001045446	0	0	0	0	0.001121101	0	0.001198365	0.001308062	0	0	0	0	0	0	0.002207213
OTU54	Coprobacillus	0	0	0.081298494	0.189989039	0.081908373	0	0	0	0	0	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU56	{Unknown Genus} Sphingomonadaceae	0.002090891	0.005093919	0.007131447	0.004175583	0.005521913	0.008968811	0	0.001198365	0	0.002582311	0.002375664	0.015751546	0.022534519	0.003790751	0.004620289	0.004414426
OTU57	Sphingomonas	0	0	0	0	0	0	0	0	0	0	0	0.006563144	0.006145778	0	0	0
OTU58	Sutterella	0.395178405	0.266157275	0.145481515	0.188945143	0.149091645	0	0	0	0	0	0	0	0.014340148	0	0	0
OTU59	Curvibacter	0	0	0	0.001043896	0.002760956	0.005605507	0	0	0.002616123	0	0	0.002625258	0.002048593	0	0	0
OTU60	{Unknown Genus} Enterobacteriaceae	0.001045446	0	0.001426289	0.001043896	0	0	0.009151747	0.001198365	0.040549909	0	0.002375664	0	0	0.012635835	0	0.001103607
OTU61	{Unknown Genus} Moraxellaceae	0	0.00127348	0.001426289	0	0	0.004484405	0	0	0	0.002582311	0.005939159	0.015751546	0.03892326	0	0.003080193	0
OTU62	Pseudomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001540096	0
OTU63	Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU64	{Unknown Family} RF39	0.003136337	0.016555237	0.018541762	0.014614541	0.00736255	0	0	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.631449092	0.639286851	1.417731629	1.740174331	1.497358685	0	0	0	0	0	0	0	0.079895112	0	0	0
	Bacteroidetes/Firmicutes	0.448	0.307	0.267	0.370	0.291	0.531	0.446	0.266	0.317	0.288	0.467	0.421	0.250	0.555	0.198	0.389

	< 28 weeks of age, Male >	Relative abundance (%)															
		Negative control (NC)		Positive control (PC)			AMP-treated - I	AMP-treated - II				AMP-treated - III					
	ID	Male - 1	Male - 2	Male - 1	Male - 2	Male - 3	Male - 1	Male - 1	Male - 3	Male - 4	Male - 6	Male - 1	Male - 2	Male - 3	Male - 4	Male - 5	Male - 6
OTU1	Curtobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU2	Frigoribacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU4	Pseudoclavibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU5	Rhodococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU6	Sanguibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU7	Bifidobacterium	6.989784366	9.649450167	1.00974887	0.799088172	0.683848797	0	6.253896995	2.094593626	5.578618458	4.210637959	1.691606305	1.735970465	6.025459008	3.724061959	0.276450657	0.620954043
OTU8	{Unknown Genus} Coriobacteriaceae	3.380349223	2.766582077	1.42062079	0.800327069	0.416953036	0	0	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.644637125	0.4416082	1.142970978	1.179429364	1.211912944	0.232133974	2.33975558	0.698197875	0.877134248	0.652277669	0.449246265	3.28098418	0.611208701	0.763247229	0.223995917	0.080345255
OTU10	{Unknown Family} Bacteroidales	0	0	0.002490133	0.003716689	0.008018328	0	0	0.005734685	0.012275416	0.017234979	0	0	0	0	0	0
OTU11	Bacteroides	0.324982352	0.299795989	1.668389009	2.610354696	1.32187858	0	0	0	0	0	0	0	0	0	0	0
OTU12	Parabacteroides	0.913679892	0.971538041	1.42062079	1.645254407	3.266895762	0	0	0	0	0	0.00110925	0.001157314	0	0	0	0
OTU13	{Unknown Genus} Rikenellaceae	0.64330523	0.798626661	2.793929056	4.835412614	3.317296678	4.034709556	0.018705574	1.63438517	1.802254213	2.892824946	6.202926202	0.644623699	1.701277763	0.261909579	10.66957767	7.864652679
OTU14	{Unknown Genus} S24-7	25.90135986	23.41767428	16.37013335	18.54504008	16.64948454	1.784115404	9.349669535	22.92296885	19.04474947	22.5433526	3.938946878	2.365549087	5.630820319	3.165396461	1.419113373	6.527478077
OTU15	Mucispirillum	0.025306003	0.007463801	0.053537856	0.117695157	0.436426117	0	0	0.001433671	0	0	0	0	0	0	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0.002290951	0	0	0	0	0	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0.026146395	0.003716689	0.003436426	0	0.038969946	0	0.004463788	0	0.0022185	0.002314627	0.123926174	0	0.001417696	0.011477894
OTU18	Jeotgalicoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU19	Staphylococcus	0.001331895	0	0.004980266	0.001238896	0.071019473	0.042005195	0	0	0	0	0	0	0	0	0	0.009182315
OTU20	Granulicatella	0.001331895	0	0.001245066	0.007433378	0.006872852	0.232133974	0.004676394	0.004301014	0.008927575	0.003977303	0.019966501	0.060180309	0.018047501	0.004496302	0.004253087	0.016069051
OTU21	{Unknown Genus} Enterococcaceae	0	0	0	0.004955586	0.002290951	0.011053999	0.003117596	0.005734685	0.004463788	0	0.0110925	0.06365225	0.007219	0.006744453	0.011341565	0.019512419
OTU22	Enterococcus	0.001331895	0.009951734	0.002490133	0.021061239	0.022909507	0.844525507	0.007793989	0.012903041	0.011159469	0.002651535	0.054353252	0.188642124	0.078205838	0.017985207	0.019847739	0.020660208
OTU23	Vagococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU24	Lactobacillus	25.84408838	27.61855003	18.49172653	21.0426552	3.524627721	3.522909412	14.73219853	13.43063182	17.962281	16.60789097	21.36859269	29.24300115	33.85229925	16.46882939	7.122503083	5.209815895
OTU25	Leuconostoc	0.001331895	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU26	Weissella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU27	Lactococcus	0	0	0.956211014	1.048106347	1.178694158	0.057480794	1.608679386	1.830798125	5.305211472	3.579572573	3.535179865	9.135833902	2.977837669	5.982329534	6.480286942	6.809834259
OTU28	Streptococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU29	Turicibacter	1.564976492	4.158580883	0.76945105	0.439808219	0.517754868	0.006632399	0.006235191	0.001433671	0.004463788	0.006628838	0.096504753	0.402745148	0.206944678	1.214001484	0.650722316	0.84018181
OTU30	{Unknown Order} Clostridia	0	0	0.003735199	0	0.002290951	0	0	0	0	0.00530307	0	0	0.002406333	0.003372226	0	0
OTU31	{Unknown Family} Clostridiales	12.61304458	11.87739464	12.56894555	15.68071162	19.81672394	36.3930802	10.96146652	10.89160012	11.84800803	9.513708437	30.36128274	9.079125534	15.05402219	14.12738023	32.67221458	26.89155686
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.045284426	0.008707767	0.033616793	0.048316959	0.049255441	0.006632399	0.023381968	0.004301014	0.037942194	0.025189585	0.0066555	0.026618214	0.004812667	0.010116679	0.008506174	0.011477894
OTU33	{Unknown Genus} Christensenellaceae	0.053275795	0.019903468	0.004980266	0.011150068	0.016036655	0	0.004676394	0.005734685	0.001115947	0	0	0	0	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.266378977	0.227645917	7.497790007	5.064608447	5.695303551	0.54275134	0.991395436	1.227222549	0.669568129	1.213077372	0.849685528	3.64322335	0.70746204	3.79038241	3.025362576	2.051099582
OTU35	02d06	0	0	0	0.002477793	0.010309278	0	0	0	0	0	0	0	0	0.002248151	0	0
OTU36	Candidatus Arthromitus	0	0	0	0	0	0	0	0	0	0	0	0	0.001203167	0	0	0
OTU37	Clostridium	0.085241273	0.250037319	0.023656262	0.01362786	0.013745704	0	0.006235191	0	0.002231894	0.002651535	0.008874	0.041663291	0.009625334	0.010116679	0.002835391	0.012625683
OTU38	SMB53	0.00266379	0	4.768604405	3.538288093	5.476517755	0.0022108	0.392817059	0.249458789	0.117174422	0.157766347	1.803640559	8.236601201	3.160719012	5.203345249	5.741667494	4.580827327
OTU39	Dehalobacterium	0.031965477	0.047270737	0.037351993	0.012388964	0.053837342	0	0	0	0	0	0.082084503	0.015045077	0.028876002	0	0	0
OTU40	Anaerofustis	0.031965477	0.019903468	0.003735199	0.003716689	0.012600229	0	0	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	2.264221308	1.591033488	1.023444601	0.773071348	2.26231386	0.763831316	0.968013468	0.807156887	1.035598706	0.608527337	1.205754789	1.251056049	1.169478066	2.872012769	2.802784354	2.303613241
OTU42	[Ruminococcus]	1.048201276	0.422948699	0.632493744	0.585997993	2.211912944	1.99856298	1.019453797	0.559131769	0.650597032	0.312881158	1.454226797	1.576261183	1.072021561	1.8817023	1.42336646	1.185666407
OTU43	Coprococcus	0.131857594	0.032343136	0.194230362	0.265123828	0.329896907	2.945890676	0.430228208	0.362718814	0.29684187	0.408336427	0.958392031	0.240721238	0.572707366	0.463119085	0.928590669	1.275193976
OTU44	Dorea	0.085241273	0.167935513	0.392195924	0.372907814	0.506300115	0.138174985	1.055306148	0.461642127	0.355987055	0.127273691	0.725449524	1.832027498	0.667757538	1.135316202	0.561407488	0.573894679
OTU45	Roseburia	0.069258534	0.009951734	0.217886624	0.2403459	0.66093929	0	0	0	0	0	0	0	0	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0.001245066	0.03097241	0.003436426	0	0	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.982938426	1.343484102	1.464198115	0.839971753	2.41580756	6.675509866	1.681942886	6.708147553	2.479633969	0.983719574	4.392630143	3.250894025	4.262819742	1.137564353	1.501339722	6.519443552
OTU48	{Unknown Genus} Peptostreptococcaceae	0	0	4.103738934	2.944856722	4.577319588	0.0033162	0.300847986	0.197846626	0.094855485	0.102084107	1.699371055	7.215850568	2.946555334	4.443470246	4.872620043	3.900188237
OTU49	{Unknown Genus} Ruminococcaceae	1.303925094	1.664427526	6.258948915	2.645043795	11.2233677	20.35372796	10.05112857	9.543949191	5.365472604	7.035848756	5.867932691	0.769613573	2.64215415	16.22490502	8.249571147	11.6156283
OTU50	Oscillospira	1.278619091	1.675623227	1.190283502	1.440836503	2.169530355	5.615431382	0.735752588	1.804992043	1.531079121	1.365540648	5.500770929	0.538150844	2.489351974	1.817629999	4.081545855	4.280106515
OTU51	Ruminococcus	1.88596316	1.222819326	0.404646588	0.407596913	0.406643757	1.880285193	0.422434219	0.669524451	0.511103671	0.416291033	2.258433073	0.822850001	1.264528238	1.346642387	1.463061939	2.330012396
OTU52	{Unknown Genus} Erysipelotrichaceae	0.146508438	0.108225108	0.503006835	0.753249006	1.053837342	11.89078649	0.913455543	0.840131324	0.537886397	0.603224267	1.519672549	4.14202553	0.671367038	1.41745914	0.891730581	0.957256324
OTU53	Allobaculum	10.17168125	8.003682142	12.41829252	11.04971691	8.325315006	0.0022108	34.63648834	16.66069304	21.44849905	19.83348359	3.541835365	9.918177925	11.74531367	12.24567793	4.818747608	3.229879253
OTU54	Coprobacillus	0	0	0.002490133	0	0.001145475	0	0	0	0	0	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU56	{Unknown Genus} Sphingomonadaceae	0.00266379	0.004975867	0.004980266	0	0.001145475	0.018791798	0.003117596	0	0.018971097	0.02784112	0.013311	0.019674332	0.002406333	0.015737056	0.022683131	0.011477894
OTU57	Sphingomonas	0	0	0	0	0	0	0	0.004301014	0	0	0	0	0	0	0.002835391	0
OTU58	Sutterella	0.352952145	0.439120267	0.002490133	0	0	0	0	0	0	0	0	0	0	0	0	0.001147789
OTU59	Curvibacter	0	0	0	0	0.001145475	0	0	0	0	0	0	0	0	0	0.001417696	0
OTU60	{Unknown Genus} Enterobacteriaceae	0	0.001243967	0.105830646	0.027255721	0.041237113	0	0.017146776	0.151969147	0.112710635	0.148485973	0.151967255	0.193271378	0.22860168	0.014612981	0.019847739	0.149212617
OTU61	{Unknown Genus} Moraxellaceae	0.001331895	0	0	0	0.002290951	0.0011054	0	0	0.007811628	0.013257676	0.00110925	0	0.001203167	0.004496302	0.001417696	0.005738947
OTU62	Pseudomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.001147789
OTU63	Stenotrophomonas	0	0.001243967	0	0	0	0	0	0	0	0	0	0.002314627	0	0	0.001417696	0.001147789
OTU64	{Unknown Family} RF39	0.003995685	0.002487934	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.903024733	0.717768821	0.002490133	0.142473085	0.017182131	0	1.021012595	6.206362633	2.260908381	6.578458928	0.225177757	0.060180309	0.061361503	0.223691014	0.025518522	0.081493044
	Bacteroidetes/Firmicutes	0.464	0.421	0.301	0.399	0.338	0.062	0.116	0.371	0.297	0.405	0.116	0.033	0.086	0.037	0.138	0.170

	< 4 weeks of age, Female >	Relative abundance (%)															
		Negative Control (NC)			Positive control (PC)				AMP-treated - I			AMP-treated - II			AMP-treated - III		
	ID	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 3	Female - 4	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 5	Female - 1	Female - 2	Female - 4
OTU1	Curtobacterium	0	0	0	0	0	0	0	0	0.001654602	0	0	0	0	0	0	0
OTU2	Frigoribacterium	0	0	0	0	0	0	0	0	0	0	0	0	0.00245468	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0	0	0	0.001654602	0	0	0	0	0	0	0
OTU4	Pseudoclavibacter	0	0	0	0	0	0	0	0	0.007445708	0.000947346	0	0	0.00245468	0.00413963	0.002171317	0.001086827
OTU5	Rhodococcus	0	0	0	0	0.001590483	0	0	0	0.003309204	0.000947346	0	0	0	0	0	0
OTU6	Sanguibacter	0	0	0	0	0	0	0	0	0	0	0	0.002044321	0	0	0	0
OTU7	Bifidobacterium	1.574948136	0.043226553	0.076011337	0.306247448	1.779749976	0.292104824	0.404680215	23.62471476	23.98428128	27.31389378	15.52495081	9.86487039	14.06900107	0	0	0.001086827
OTU8	{Unknown Genus} Coriobacteriaceae	0.058922456	0.104978773	0.014171605	0.012249898	0.16222922	0.035052579	0.033430105	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.592907209	0.439984562	0.2460706	0.110249081	0.862041543	0.367217493	0.380047506	0	0.000827301	0	0	0	0	0	0	0
OTU10	{Unknown Family} Bacteroidales	0	0.003087611	0.001288328	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU11	Bacteroides	0.166946958	0.196063296	0.316928627	0.17558187	0.785698381	0.904690369	0.028151667	0	0	0	0	0	0	0	0.001085658	0
OTU12	Parabacteroides	0.022095921	0.015438055	0.045091471	0.220498163	0.270382034	0.065097646	0.003518958	0	0	0	0	0	0	0	0	0
OTU13	{Unknown Genus} Rikenellaceae	2.200999227	3.657275183	5.014171605	4.499795835	1.461653466	3.228175597	0.765373449	0	0.003309204	0.001894693	0.005961963	0.002044321	0	0	0	0
OTU14	{Unknown Genus} S24-7	20.84013601	22.01157854	12.87683587	11.759902	20.54108216	23.64880654	6.408023225	0.00175531	0.014064116	0.002842039	0.002980981	0.00102216	0	0	0.003256975	0
OTU15	Mucispirillum	0.077335723	0.126592049	0.325946921	1.449571254	0.112924261	0.180270406	0.809360429	0	0.000827301	0	0	0	0	0	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0	0	0	0	0	0.071050986	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU18	Jeotgalicoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU19	Staphylococcus	0	0	0.009018294	0.008166599	0.011133378	0	0.001759479	4.135509918	0.685832472	4.18537676	0	0	0	0	0	0
OTU20	Granulicatella	0.002455102	0.004631416	0.010306622	0.004083299	0.399211121	0.00166917	0.049265417	0.012287169	0.000827301	0.001894693	7.151374232	5.187464224	4.348466438	19.12301945	16.42601238	12.75390986
OTU21	{Unknown Genus} Enterococcaceae	0	0.006175222	0.003864983	0	0.007952413	0	0	0	0	0	5.160078698	1.545506583	2.318445696	0	0	0
OTU22	Enterococcus	0.008592858	0.026244693	0.009018294	0.024499796	1.331233896	0.021699215	0.147796252	0.038616816	0.010754912	0.009473465	23.31127407	18.79344182	15.06069197	72.10717501	69.51796765	48.11163883
OTU23	Vagococcus	0	0	0	0	0	0	0	0	0	0	0.005961963	0	0.003682021	0.009314167	0.009770926	0.013041919
OTU24	Lactobacillus	45.27454182	50.53338479	11.66451945	16.71702736	41.90444381	28.97012185	36.55493974	0.289626119	0.033919338	0.008526118	14.5203601	35.14289803	42.14931821	0.012418889	0.010856585	0.00326048
OTU25	Leuconostoc	0	0	0	0.004083299	0.009542895	0.006676682	0	0	0	0	0	0	0	0	0	0
OTU26	Weissella	0	0	0	0	0	0	0	0.00351062	0	0.001894693	0	0	0	0	0	0
OTU27	Lactococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU28	Streptococcus	0.002455102	0	0	0	0.001590483	0.008345852	0.005278438	0.012287169	0.010754912	0.003789386	0	0	0.00122734	0.002069815	0.004342634	0
OTU29	Turicibacter	3.083608509	4.36896951	1.034527184	0	0.003180965	0	0.005278438	71.84131999	75.15698035	68.31978628	14.69921898	14.69253414	12.19607988	8.726339429	13.79220497	39.04424471
OTU30	{Unknown Order} Clostridia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU31	{Unknown Family} Clostridiales	11.71083805	8.336549595	38.77351198	28.46876276	12.69205077	20.92638958	33.40195302	0.010531859	0.020682523	0.01894693	0.005961963	0.005110802	0.004909361	0	0.007599609	0.024997011
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.015958165	0.023157082	0.088894615	0.036749694	0.015904826	0.068435987	0.103809272	0	0	0	0	0	0	0	0	0
OTU33	{Unknown Genus} Christensenellaceae	0.025778574	0.012350444	0.064416388	0.020416497	0.063619302	0.016691704	0.012316354	0	0	0	0	0	0	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.163264304	0.120416827	0.159752641	0.11841568	0.252886726	0.090135203	0.056303334	0	0.000827301	0	0	0	0	0.001034907	0	0
OTU35	02d06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU36	Candidatus Arthromitus	4.915114837	2.429949826	3.272352486	0.445079624	4.916181569	1.782674011	0.892055951	0	0	0	0	0	0	0.001034907	0	0
OTU37	Clostridium	0	0	0	0.685994283	0.138371982	0.964780504	0.026392188	0	0	0	0	0	0	0	0	0
OTU38	SMB53	0.001227551	0.063296025	0	0	0	0	0	0	0	0	0	0	0.00122734	0	0	0
OTU39	Dehalobacterium	0.041736739	0.01698186	0.118526153	0.167415272	0.058847854	0.206977132	0.102049793	0	0	0	0	0	0	0	0	0
OTU40	Anaerofustis	0.073653069	0.023157082	0.015459933	0.048999592	0.009542895	0.04006009	0.02287323	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	2.343395161	1.869548437	8.405050245	10.18783177	2.533638706	5.69354031	7.836720331	0.00175531	0.008273009	0.002842039	0	0.00102216	0	0.001034907	0	0.004347306
OTU42	[Ruminococcus]	0.978358273	0.798147433	2.3537748	2.278481013	2.28075198	1.313637122	1.212281165	0	0.002481903	0.000947346	0.026828832	0	0	0	0.001085658	0
OTU43	Coprococcus	0.117844911	0.061752219	0.644163875	1.910984075	0.047714477	0.542480387	0.422275007	0	0.001654602	0	0.002980981	0	0	0	0.001085658	0
OTU44	Dorea	0.06383266	0.058664608	0.307910332	0.191915067	0.919298915	0.185277917	0.200580628	0	0	0	0	0	0	0	0	0
OTU45	Roseburia	0.02086837	0.024700888	0.213862407	0	0	0.033383408	0.788246679	0	0	0	0	0	0	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0	0	0	0	0.019354271	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.606410272	1.269008105	0.421283174	0.387913434	0	0.872976131	0.2621624	0.00351062	0	0	0	0	0	0.001034907	0	0
OTU48	{Unknown Genus} Peptostreptococcaceae	0	0.038595137	0	0	0	0	0	0.00175531	0.000827301	0	0	0	0.00122734	0	0	0
OTU49	{Unknown Genus} Ruminococcaceae	2.583995188	2.062524122	7.574078846	10.88607595	3.141203041	3.530295443	4.111902877	0.00351062	0.004136505	0.005684079	0.002980981	0	0	0.001034907	0.001085658	0.001086827
OTU50	Oscillospira	1.514798129	0.694712466	3.291677403	5.287872601	2.024684289	2.702386914	3.596375473	0.00175531	0.000827301	0	0	0	0	0	0.001085658	0.001086827
OTU51	Ruminococcus	0.642009256	0.168274797	2.062612729	2.462229481	0.566211789	0.762810883	1.055687516	0	0.001654602	0.001894693	0	0	0	0	0.001085658	0
OTU52	{Unknown Genus} Erysipelotrichaceae	0.175539816	0.211501351	0.398093275	0.383830135	0.45487801	0.617593056	0.212896982	0	0	0.000947346	0	0	0	0.001034907	0.001085658	0.001086827
OTU53	Allobaculum	0	0	0	0	0.001590483	0	0	0	0.001654602	0	0	0	0	0.001034907	0.001085658	0.00326048
OTU54	Coprobacillus	0	0	0	0	0	0.273743949	0.007037917	0	0	0.000947346	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0	0	0.00175531	0	0.000947346	0	0	0	0.002069815	0	0.00326048
OTU56	{Unknown Genus} Sphingomonadaceae	0.01104796	0	0.005153311	0	0	0.005007511	0.003518958	0.00351062	0.019027921	0.019894276	0.017885888	0.004088642	0.009818722	0.005174537	0.128107697	0.015215572
OTU57	Sphingomonas	0	0	0	0	0	0.003338341	0	0	0.005791107	0.001894693	0	0	0.00245468	0	0.011942243	0.002173653
OTU58	Sutterella	0.009820409	0.06483983	0.003864983	0.040832993	0.047714477	0.140210315	0.015835313	0	0	0	0	0	0	0	0	0
OTU59	Curvibacter	0	0	0	0	0.001590483	0.00166917	0	0	0.002481903	0.003789386	0	0.002044321	0	0	0.015199218	0.00326048
OTU60	{Unknown Genus} Enterobacteriaceae	0.008592858	0.050945581	0.018036589	0.016333197	0.097019436	0.053413454	0.019354271	0.00175531	0.000827301	0.001894693	19.56119955	14.75284161	9.822403869	0	0	0
OTU61	{Unknown Genus} Moraxellaceae	0.003682653	0	0.009018294	0	0.003180965	0.00166917	0.005278438	0.007021239	0.011582213	0.01610489	0	0.003066481	0.006136701	0.001034907	0.058625556	0.011955092
OTU62	Pseudomonas	0	0	0	0	0	0	0	0.00175531	0	0.000947346	0	0	0	0	0.003256975	0
OTU63	Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU64	{Unknown Family} RF39	0.017185716	0.003087611	0.005153311	0.004083299	0	0.018360875	0	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.049102046	0.060208414	0.145581036	0.677827685	0.08747654	1.4221332	0.015835313	0.00175531	0.000827301	0	0	0	0	0	0	0
	Bacteroidetes/Firmicutes	0.312	0.353	0.226	0.206	0.313	0.400	0.079	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	< 8 weeks of age, Female >	Relative abundance (%)															
		Negative Control (NC)			Positive control (PC)				AMP-treated - I			AMP-treated - II			AMP-treated - III		
	ID	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 3	Female - 4	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 5	Female - 1	Female - 2	Female - 4
OTU1	Curtobacterium	0	0	0.004063224	0	0	0	0	0	0	0.003221649	0	0	0	0	0	0
OTU2	Frigoribacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU4	Pseudoclavibacter	0	0	0	0	0	0	0	0.002366304	0	0	0	0	0	0	0	0
OTU5	Rhodococcus	0	0	0	0	0	0	0	0	0	0	0	0	0.001201071	0	0.001492537	0
OTU6	Sanguibacter	0	0	0	0	0	0	0	0.002366304	0	0	0	0	0	0	0	0
OTU7	Bifidobacterium	0	0	0.005417632	3.761772853	1.349857116	5.831716406	1.936388202	0.009465215	0.007191313	0.004295533	0.001172415	0	0.003603214	0.011854311	0.021641791	0.008287522
OTU8	{Unknown Genus} Coriobacteriaceae	0.793288848	0.300632271	0.570205735	0.995844875	0.719445123	1.829437309	1.455181284	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.372041669	0.275017863	0.353500467	0.502770083	0.775449833	0.759185685	0.537564486	0.333648841	0.860560443	0.527276632	0.350552208	0.359891223	0.571709965	0.356617175	0.448507463	0.219619327
OTU10	{Unknown Family} Bacteroidales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU11	Bacteroides	0.093610484	0.095717001	0.1367952	0.480609418	0.061748783	0.203270812	0.184968425	0	0	0	0	0	0	0	0	0
OTU12	Parabacteroides	0.390043685	0.462407485	0.702937711	0.501385042	0.213966713	0.084696172	0.661840147	0	0.001198552	0	0	0	0	0	0.000746269	0
OTU13	{Unknown Genus} Rikenellaceae	0.512457395	1.48968009	0.935895873	1.247922438	0.733805305	0.291046845	0.713862516	5.386890677	7.162547642	10.19544674	3.531315216	4.043279523	3.15401338	5.876774442	5.102985075	3.347468162
OTU14	{Unknown Genus} S24-7	19.62819836	9.721342195	16.9287446	23.9265928	22.84992174	19.79580523	22.4259765	19.62375769	28.08447396	15.50579897	20.55126973	15.18254933	24.0430464	17.34088058	25.4761194	11.3228266
OTU15	Mucispirillum	0.146416399	1.772786713	0.556661655	0.558171745	0.226890877	0.007699652	0.791896071	0	0	0	0	0	0	0.000987859	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU18	Jeotgalicoccus	0.002400269	0	0.002708816	0	0	0	0	0.016564127	0.059927607	0.024699313	0	0	0	0	0.001492537	0
OTU19	Staphylococcus	0	0	0	0	0.001436018	0	0	0.00591576	0.005992761	0	0	0	0	0	0	0
OTU20	Granulicatella	0	0.001348127	0	0.001385042	0.064620819	0.013859374	0	0.002366304	0.001198552	0.003221649	0.007034492	0.005786033	0.009608571	0	0.000746269	0
OTU21	{Unknown Genus} Enterococcaceae	0	0	0	0	0	0	0.001445066	0	0.007191313	0.004295533	0.011724154	0.001157207	0.01801607	0	0.002238806	0
OTU22	Enterococcus	0.001200134	0.005392507	0.004063224	0.023545706	0.277151514	0.052357633	0.014450658	0.011831519	0.003595656	0.008591065	0.048069032	0.011572065	0.015613928	0.006915014	0.005970149	0.00207188
OTU23	Vagococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU24	Lactobacillus	18.6356872	11.89452256	13.81496079	25.25484765	22.24392205	47.39905756	22.40285545	41.65522953	28.87911403	16.03737113	53.59579806	30.51090667	42.62482134	10.71234528	38.02238806	13.69443907
OTU25	Leuconostoc	0	0.001348127	0.002708816	0	0	0	0	0.004732608	0	0.001073883	0	0.001157207	0.004804285	0	0	0
OTU26	Weissella	0	0	0	0	0	0	0	0	0.002397104	0	0.001172415	0	0	0	0	0.001381254
OTU27	Lactococcus	0	0	0	0	0	0.00153993	0.001445066	0	0	0	0	0	0	0	0.002238806	0.00207188
OTU28	Streptococcus	0	0	0.004063224	0.001385042	0	0.003079861	0.001445066	0.004732608	0.002397104	0.006443299	0.003517246	0	0.001201071	0.002963578	0.005223881	0.001381254
OTU29	Turicibacter	1.155729442	0.912681829	1.825741877	4.638504155	0.933411836	8.059995688	4.952240575	4.111452911	3.867727785	4.146262887	3.027176589	2.777295608	6.490589606	0.328957117	1.320895522	1.031105832
OTU30	{Unknown Order} Clostridia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU31	{Unknown Family} Clostridiales	34.8026979	47.96095825	36.38616879	23.00831025	25.3787498	5.670023715	24.37826043	15.85541884	17.19922334	31.55068729	10.18594508	29.53885321	13.40395633	39.14490907	14.28059701	43.42868588
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.108012097	0.146945819	0.09751737	0.042936288	0.107701366	0.007699652	0.070808225	0.004732608	0.017978282	0.015034364	0.009379323	0.020829717	0.010809642	0	0	0
OTU33	{Unknown Genus} Christensenellaceae	0.019202151	0.010785014	0.016252895	0.01800554	0.011488146	0.010779513	0.013005592	0	0	0	0	0	0	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.150016802	0.129420171	0.181490661	0.05401662	0.103393311	0.063137146	0.124275661	0.11713204	0.303233694	0.093427835	0.001172415	0	0.003603214	0.001975718	0.007462687	0.004143761
OTU35	02d06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU36	Candidatus Arthromitus	0.019202151	0.006740634	0.023024935	0.001385042	0.011488146	0.00153993	0.002890132	0	0	0	0	0	0	0.000987859	0	0
OTU37	Clostridium	0.108012097	0	0.138149608	0.139889197	0.08041702	0.192491299	0.037571711	0.556081401	0.692763142	0.792525773	0.008206908	0.108777411	0.054048211	0.060259412	0.589552239	0.111881544
OTU38	SMB53	0.171619221	0.080887607	0.092099739	0.001385042	0	0.003079861	0	0	0	0	0	0	0	0	0	0
OTU39	Dehalobacterium	0.267629975	0.350512962	0.411740008	0.217451524	0.356132516	0.030798608	0.21097961	0	0	0	0	0	0	0	0	0
OTU40	Anaerofustis	0.034803898	0.032355043	0.027088158	0.01800554	0.0157962	0.032338538	0.028901316	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	6.484326245	7.591301886	7.523735999	3.534626039	6.644456252	1.615386985	4.326527073	5.016564127	5.567274732	8.35266323	3.094004268	7.067060117	3.620029066	11.5134991	5.767910448	12.84565872
OTU42	[Ruminococcus]	3.421583217	2.608625315	2.611298471	1.576177285	4.641210851	0.890079768	2.810653025	1.24704212	1.145815854	3.029424399	0.732759631	2.283168431	1.701918111	3.470349406	0.988059701	2.673416393
OTU43	Coprococcus	0.531659546	0.589131402	0.400904744	0.245152355	0.255611241	0.064677077	0.299128625	0.847136772	0.538149915	1.275773196	0.220414097	0.957009778	0.266637841	1.029349297	0.468656716	1.476560126
OTU44	Dorea	0.432048389	0.285802877	0.350791651	0.105263158	0.334592243	0.132434014	0.2283204	0	0	0	0	0	0	0.071125863	0.002238806	0.001381254
OTU45	Roseburia	0.141615861	0.239966566	0.138149608	0.008310249	0.034464437	0.020019095	0.270227309	0	0	0	0	0	0	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0	0.013850416	0.001436018	0.004619791	0.020230922	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.524458739	0.614745811	0.784202186	0.490304709	0.485374155	0.626751671	0.609817777	1.534548036	1.976412494	1.347723368	0	0.001157207	0	0.401070839	1.3	0.391585403
OTU48	{Unknown Genus} Peptostreptococcaceae	0.140415727	0.068754466	0.081264475	0.001385042	0.001436018	0	0	0	0.001198552	0	0	0	0	0	0	0
OTU49	{Unknown Genus} Ruminococcaceae	2.675099611	4.482521536	4.231170344	2.718836565	4.14434855	1.13030891	3.91612838	1.347610033	1.654001966	2.891967354	1.166553333	3.331597524	1.383634202	4.153947979	2.55	3.627172021
OTU50	Oscillospira	2.789112381	4.250643731	4.519659231	2.44598338	3.602969686	1.330499861	3.440701724	1.67297681	1.247692787	3.107817869	1.023518653	1.741595788	1.05454065	3.774610043	2.489552239	3.647890826
OTU51	Ruminococcus	4.424895588	2.91195384	4.850134764	1.337950139	1.639932794	0.674489513	1.552000694	0.553715097	0.646019608	1.00193299	0.757380355	1.146791645	0.747066383	1.604283358	0.97761194	2.060139783
OTU52	{Unknown Genus} Erysipelotrichaceae	0.561662906	0.407134287	0.702937711	0.577562327	0.386288898	0.412701346	0.258666782	0.020113583	0.046743534	0.049398625	1.389312261	0.843603541	0.778294238	0.061247271	0.142537313	0.040056355
OTU53	Allobaculum	0	0	0	0.002770083	0.002872036	0.010779513	0.007225329	0.001183152	0.001198552	0	0	0	0	0	0	0.000690627
OTU54	Coprobacillus	0	0	0	0.03601108	0.366184643	0.221749977	0.132946056	0	0	0	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU56	{Unknown Genus} Sphingomonadaceae	0.04080457	0.035051296	0.021670527	0.001385042	0.018668237	0.010779513	0.005780263	0.021296735	0	0.006443299	0.001172415	0.002314413	0	0.06223513	0.01641791	0.050415757
OTU57	Sphingomonas	0	0	0.004063224	0	0	0	0.001445066	0.003549456	0	0.002147766	0	0.001157207	0	0.000987859	0	0.00207188
OTU58	Sutterella	0.180020162	0.16716772	0.319640269	0.354570637	0.170886167	0.455819397	0.270227309	0	0	0	0	0	0	0	0	0
OTU59	Curvibacter	0.002400269	0	0	0	0	0.00153993	0.001445066	0.001183152	0	0.001073883	0	0	0	0	0	0.000690627
OTU60	{Unknown Genus} Enterobacteriaceae	0	0.001348127	0	0.01800554	0.113445438	0.003079861	0	0.011831519	0.014382626	0.007517182	0.280207283	0.055545912	0.037233212	0.000987859	0.004477612	0
OTU61	{Unknown Genus} Moraxellaceae	0.008400941	0.005392507	0	0	0	0	0.002890132	0.016564127	0.001198552	0.006443299	0.001172415	0.006943239	0	0.009878592	0.001492537	0.006906268
OTU62	Pseudomonas	0.001200134	0	0	0.001385042	0	0	0	0	0	0	0	0	0	0	0	0
OTU63	Stenotrophomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU64	{Unknown Family} RF39	0.002400269	0.009436887	0.001354408	0.013850416	0.011488146	0	0.004335197	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.22562527	0.07953948	0.237021386	1.120498615	0.627539957	2.049647356	0.893050678	0	0.001198552	0	0	0	0	0	0.000746269	0
	Bacteroidetes/Firmicutes	0.266	0.138	0.236	0.393	0.331	0.297	0.342	0.335	0.552	0.349	0.320	0.239	0.377	0.304	0.444	0.173

	< 28 weeks of age, Female >	Relative abundance (%)															
		Negative Control (NC)			Positive control (PC)				AMP-treated - I			AMP-treated - II			AMP-treated - III		
	ID	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 3	Female - 4	Female - 1	Female - 2	Female - 3	Female - 1	Female - 2	Female - 5	Female - 1	Female - 2	Female - 4
OTU1	Curtobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU2	Frigoribacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU3	Leucobacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU4	Pseudoclavibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU5	Rhodococcus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU6	Sanguibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU7	Bifidobacterium	15.99582872	14.4739103	12.08776267	0.067281336	0.116821037	0.259210171	0.045765936	0.064261556	0.998683055	0.142829841	0.176943118	0.668799069	0.279259848	0.738586405	0.584765892	2.735846793
OTU8	{Unknown Genus} Coriobacteriaceae	1.163633589	2.361246349	2.78986403	0	0	0.001172897	0	0	0	0	0	0	0	0	0	0
OTU9	Adlercreutzia	0.319204405	0.416690532	0.625463535	1.335659108	1.631918368	1.493097503	1.690479255	0.974069899	2.568042142	0.935434877	0.835866261	2.336725792	1.797025769	0.415285763	1.004468779	0.831553433
OTU10	{Unknown Family} Bacteroidales	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU11	Bacteroides	0.057227881	0.090211352	0.033374536	4.100423623	9.984622537	4.607138249	4.919838103	0	0	0	0	0	0	0	0	0
OTU12	Parabacteroides	0.063586535	0.131737213	0.030902349	10.98305507	8.814028061	6.916571858	13.59534332	0	0	0	0	0	0	0	0	0
OTU13	{Unknown Genus} Rikenellaceae	0.461638243	0.121713729	0.0815822	2.30127087	1.712977864	4.161437502	1.341513994	4.776775648	0.149985367	1.679759402	11.33738602	2.504216342	11.35770235	1.993912749	1.353717944	2.545057477
OTU14	{Unknown Genus} S24-7	17.53970979	24.3284266	25.815822	4.203837528	4.328338638	11.09091122	11.41431044	11.8951522	1.031606673	9.166255947	8.62244898	7.287002035	10.47678511	12.45045654	10.42211844	11.74134248
OTU15	Mucispirillum	0.005086923	0	0	0.573137304	0.8511247	0.11025229	0.224539123	0	0	0	0	0	0	0	0	0
OTU16	{Unknown Genus} Planococcaceae	0	0	0	0	0.002384103	0	0	0	0	0	0	0	0	0	0	0
OTU17	Sporosarcina	0	0	0	0.003737852	0	0.002345793	0.005720742	0	0	0	0	0	0	0.001352722	0.00603889	0.002399866
OTU18	Jeotgalicoccus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU19	Staphylococcus	0	0	0.001236094	0.026164964	0.023841028	0.052780352	0.017162226	0.02367531	0.029265438	0.025146099	0	0	0	0	0.010064817	0
OTU20	Granulicatella	0	0	0	0.54697234	0.714038789	0.077411182	0.032894266	0.004509583	0.010974539	0.001005844	0.016283109	0.020936319	0.027244863	0.033818059	0.102661138	0.004799731
OTU21	{Unknown Genus} Enterococcaceae	0	0	0	0.022427112	0.057218467	0.004691587	0.005720742	0	0.00365818	0	0.004342162	0.016283803	0.007946418	0.001352722	0.007045372	0
OTU22	Enterococcus	0.003815192	0	0	2.143035136	3.03734697	0.281495209	0.098682799	0.03156708	0.007316359	0.004023376	0.07273122	0.052340797	0.157793166	0.155563071	0.486130682	0.00839953
OTU23	Vagococcus	0	0	0	0.003737852	0.001192051	0	0	0	0	0	0	0	0	0	0	0
OTU24	Lactobacillus	41.14811847	33.5772381	34.48702101	8.382756043	7.885420019	21.03121078	9.573661704	3.977452086	12.78533802	7.406029029	3.585540599	6.218086653	5.311613123	24.90361853	16.15000604	9.316278288
OTU25	Leuconostoc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU26	Weissella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU27	Lactococcus	0	0	0	3.077498131	0.730727509	1.923550593	2.213927146	4.455467869	2.809482002	3.975095304	3.547546678	3.374236697	7.827222159	3.421034833	1.815693063	6.38964218
OTU28	Streptococcus	0	0.002863852	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU29	Turicibacter	4.059364389	6.969184948	1.037082818	0.084724645	0.060794621	0.186490576	0.124426138	0.013528749	0.065847234	0.015087659	0.069474598	0.411747601	0.31445113	0.018938113	0.031200934	1.14713576
OTU30	{Unknown Order} Clostridia	0	0	0	0	0	0	0	0	0	0	0	0.002326258	0.003405608	0	0.002012963	0.002399866
OTU31	{Unknown Family} Clostridiales	5.622321417	3.702961223	4.482076638	23.62945427	24.32380884	12.37406022	15.26866035	19.6832018	13.13652327	13.95206148	34.51801997	14.40767665	12.92201158	11.89854582	14.60002416	13.44044733
OTU32	{Unknown Genus} [Mogibacteriaceae]	0.011445576	0.004295779	0.017305315	0.032394717	0.035761542	0.037532694	0.014301855	0.004509583	0	0.01005844	0.004342162	0.009305031	0.004540811	0	0.001006482	0.001199933
OTU33	{Unknown Genus} Christensenellaceae	0.027978075	0.027206598	0.049443758	0.016197359	0.064370776	0.008210277	0.005720742	0	0	0	0.028224056	0.024425705	0.007946418	0	0	0
OTU34	{Unknown Genus} Clostridiaceae	0.066129996	0.061572828	0.060568603	1.518813855	2.055096616	3.1304613	1.817765764	1.107102593	3.947175885	1.196954305	1.237516283	2.772899099	5.191281644	0.250253635	1.925399573	4.246562193
OTU35	02d06	0	0	0	0	0	0	0	0	0	0	0.004342162	0.004652515	0.003405608	0	0.004025927	0.001199933
OTU36	Candidatus Arthromitus	0.024162883	0.024342746	0.00618047	0	0	0	0	0.001127396	0	0	0	0	0	0	0	0
OTU37	Clostridium	0.045782305	0.081619795	0.061804697	0.004983803	0.001192051	0.001172897	0	0.003382187	0.007316359	0.013075971	0.002171081	0.012794417	0.002270405	0	0.012077781	0.016799059
OTU38	SMB53	0.001271731	0	0	3.587091951	2.043176102	3.225465933	2.75882782	0.456595265	1.29133743	1.202989368	0.795701259	2.929921489	5.77137019	0.240784579	1.357743871	3.349012455
OTU39	Dehalobacterium	0.029249806	0.018615041	0.018541409	0.07475704	0.010728463	0.032841108	0.064358347	0.036076663	0.036581797	0.133777246	0	0	0	0	0	0
OTU40	Anaerofustis	0.015260768	0.02291082	0.017305315	0.002491901	0.001192051	0.00351869	0	0	0	0	0	0	0	0	0	0
OTU41	{Unknown Genus} Lachnospiraceae	1.861813742	0.704507704	0.939431397	1.034139048	0.880925986	0.646266083	0.583515682	0.843291995	1.068188469	1.039036804	3.771168042	3.377726083	1.706209558	1.078119716	0.682394621	1.181933812
OTU42	[Ruminococcus]	0.5468442	0.389483934	0.781211372	3.062546723	2.524764868	1.197527534	1.187053961	1.445321308	1.331577407	0.90324787	1.639166305	1.487641756	1.491656261	1.029421711	0.961190064	1.385922388
OTU43	Coprococcus	0.09156461	0.032934303	0.050679852	0.713929728	0.61271442	0.252172791	0.653594771	0.66854566	0.384108867	0.830827105	1.011723838	0.742076185	0.432512203	1.939803855	0.511292725	1.196333005
OTU44	Dorea	0.132259993	0.055845123	0.143386897	1.369299776	1.398276294	0.810471622	0.583515682	1.289740699	2.304653205	3.006467577	0.881458967	1.836580401	0.664093541	1.324315184	1.497644833	0.845952627
OTU45	Roseburia	0.030521537	0.020046967	0.033374536	1.311986045	0.56145621	0.578238075	1.209936929	0	0	0	0.677377334	0.740913056	0.179362016	0	0	0
OTU46	{Unknown Genus} Peptococcaceae	0	0	0	0	0	0.011728967	0.002860371	0	0	0	0	0	0	0	0	0
OTU47	Rc4-4	0.180585759	1.353170285	0.798516687	2.871916272	1.964500709	3.645362953	4.17471146	7.981961669	3.819139596	6.774359026	0	0	0	6.14947582	2.206207979	5.910868991
OTU48	{Unknown Genus} Peptostreptococcaceae	0	0.001431926	0	3.092449539	1.78688505	2.732849318	2.15242917	0.387824126	1.035264852	1.040042648	0.700173686	2.489095667	4.709955727	0.23537369	1.301380893	3.091026902
OTU49	{Unknown Genus} Ruminococcaceae	0.743962459	0.335070737	0.73053152	6.994767007	7.040255576	6.521305669	5.25736188	16.94588501	12.61340357	9.349319547	15.29960921	12.87816226	16.6806675	11.55901251	10.95756673	8.937099522
OTU50	Oscillospira	0.782114379	0.372300819	0.786155748	0.904560179	1.448342453	0.699046435	0.693639965	2.113866967	1.236464735	1.972459993	2.072297004	2.055248619	1.310023839	4.087926953	3.923265832	3.369411313
OTU51	Ruminococcus	0.78338611	0.592817458	0.796044499	0.35883379	0.712846738	0.276803622	0.258863575	0.804960541	0.336552531	0.976674479	1.01498046	0.796743239	0.450675446	1.20662834	0.788075204	1.2839281
OTU52	{Unknown Genus} Erysipelotrichaceae	0.096651533	0.085915574	0.378244747	2.586593571	2.20291099	1.537667578	2.143848057	2.552423901	3.350892596	5.647813798	3.153495441	5.799360279	3.108184811	3.657761245	2.617859012	1.981089059
OTU53	Allobaculum	7.023768647	8.976745518	11.07416564	7.430849738	8.278796982	7.697721062	7.838846698	5.455467869	19.87854843	6.543014917	2.370820669	7.331200931	5.531842434	5.290497125	4.336929828	6.498836065
OTU54	Coprobacillus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU55	Methylobacterium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU56	{Unknown Genus} Sphingomonadaceae	0	0.010023484	0.001236094	0.032394717	0.003576154	0.00703738	0.008581113	0.016910936	0	0.014081815	0.009769865	0	0.006811216	0.050050727	0.020129635	0.002399866
OTU57	Sphingomonas	0	0	0	0.001245951	0	0.001172897	0	0	0	0	0	0	0	0	0	0
OTU58	Sutterella	0.503605357	0.441033278	0.635352287	0.513331672	1.742779149	0.767074444	1.021152443	0	0	0	0	0	0	0	0	0
OTU59	Curvibacter	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU60	{Unknown Genus} Enterobacteriaceae	0	0	0.001236094	0.024919013	0.091787958	0.046915868	0.012871669	1.735062007	3.800848698	1.50373671	0.30720799	0.018610061	0.136224316	1.880284072	11.27058255	0.443975137
OTU61	{Unknown Genus} Moraxellaceae	0	0	0.001236094	0.002491901	0	0	0	0	0	0	0	0	0.001135203	0.009469056	0.005032409	0
OTU62	Pseudomonas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU63	Stenotrophomonas	0.001271731	0.002863852	0	0.001245951	0	0	0.001430185	0	0	0	0.001085541	0	0	0.005410889	0.005032409	0.001199933
OTU64	{Unknown Family} RF39	0	0.002863852	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OTU65	Akkermansia	0.560833238	0.206197377	1.145859085	0.970595564	0.261059257	1.557606822	6.982165587	10.25028185	9.961223295	20.53933353	2.230785931	17.39226519	2.127369735	3.972945553	9.039212529	8.089946963
	Bacteroidetes/Firmicutes	0.286	0.430	0.457	0.288	0.353	0.388	0.532	0.237	0.014	0.164	0.261	0.140	0.296	0.184	0.178	0.194