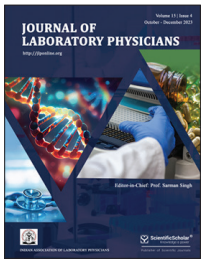
https://jlabphy.org/

Journal of Laboratory Physicians



Original Article

Short term antibiotic effects on gut microbiome in Indian preschoolers: A 16S rRNA analysis

Neha[1], Ashish Bavdekar[2], Anand Kawade[3], Krishna Chaitanya Veligandla[1], Devesh Kumar Joshi[4], Rahul Rathod[1], Bhavesh Kotak[1]

[1]Department of Medical Affairs, Dr. Reddy's Laboratories, Hyderabad, Telangana, [2]Department of Paediatrics, King Edward Memorial Hospital, Pune, Maharashtra, [3]Department of Paediatrics, Shirdi Saibaba Rural Hospital, Rural Health Program, King Edward Memorial Hospital, Pune, Maharashtra, [4]Department of Medical Affairs, Dr. Reddy's Laboratory Ltd., Hyderabad, Telangana, India.

***Corresponding author:**
Dr. Neha,
Department of Medical Affairs,
Dr. Reddy`s Laboratories,
Hyderabad, Telangana, India.

neha2@drreddys.com

Received: 11 October 2023
Accepted: 06 November 2023
EPub Ahead of Print: 29 December 2023
Published: 31 May 2024

DOI
10.25259/JLP_6_2023

Quick Response Code:

ABSTRACT

Objectives: Antibiotic use is associated with dysbiosis of the gut microbiome. The objective of this study is to investigate the effect of antibiotics on gut composition in children aged 3–5 years receiving antibiotics compared to children who did not receive antibiotics.

Materials and Methods: A total of 54 participants aged 3–5 years were included in this multi-centric cohort study. Participants were divided into two equal groups, that is the treatment-experienced group (Group 1, $n = 27$, antibiotic) and the treatment-not-experienced group (Group 2, $n = 27$, non-antibiotic). Stool samples of study participants were collected on days 0 and 5 (± 1 day) and analyzed using 16Svedberg ribosomal ribonucleic acid (16S rRNA) gene sequencing.

Statistical Analysis: The Kruskal-Wallis H-test and Benjamini-Hochberg FDR correction were applied to determine the differentially abundant pathways across the zones using Statistical Analysis of Metagenomic Profiles (STAMP) (v2.1.3).

Results: A non-significant increase in the mean abundance of the Phyla *Bacteroidota*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobiota* was observed in both groups from day 0 to day 5. An alteration in the *Firmicutes*/*Bacteroidetes* ratio was observed. A significant ($P < 0.05$) abundance of genus *Enterobacteriaceae*, *Enterococcaceae*, and *Lactobacillaceae* was observed in the participants with antibiotic treatment. The relative abundance of families *Enterococcus*, *Lactobacillus*, *Sellimonas*, *Ruminococcus*, *Torques*, and *Eggerthella* groups was observed to be significantly higher ($P < 0.05$) in participants with antibiotic treatment. Beta-diversity indices revealed significant differences at group and subgroup levels regarding the bacterial counts.

Conclusions: It was observed that a short-term course of 5 days of antibiotic usage is associated with altered microbial abundance and diversity.

Keywords: Antibiotics, Dysbiosis, Early childhood, Gene sequencing, Gut microbiomes, Stool samples

INTRODUCTION

The oral cavity, skin, and intestine of humans are inhabited by 1000 trillion microbiomes.[1] The collection of microbiomes colonizing the gut is known as "gut microbiota"[2] that forms a densely populated "mini ecosystem."[3] It is a microbial ecosystem where a diverse group of organisms live in close proximity to each other, interacting and influencing complex changes. Out of 55 phyla in

the human body, the majority of the gut bacteria belong to the *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria,* and *Verrucomicrobia*.[4] An important characteristic feature of the intestinal microbiome is the composition of microbiota and the metagenome that remains largely unaffected irrespective of the type of food intake.[3] Gut microbes are gaining importance due to their functions of stimulating host immune development, nutrient metabolism, differentiation of mucosal structure,[3] maintaining intestinal mucosal barrier by enhancing gut integrity, formation of the intestinal epithelium, and protection against pathogens, providing anti-inflammatory signals to the host,[2] and are necessary for the maintenance of intestinal homeostasis.[5]

The role of microbiomes is crucial during early life as the changes in relation to the composition and the abundance of microbiomes become more or less stable, remain the same throughout life, and dictate the health of the host.[5] Various internal and external factors influence the gut microbiota, starting from birth to adulthood.[3] The human fecal microbiota consists of four main groups of bacteria (phyla), that are *Firmicutes*, *Bacteroidetes*, *Proteobacteria,* and *Actinobacteria*, of which the first two bacteria account for more than 80% of the microbiota. *Firmicutes* comprise mostly of Gram-positive bacteria, while *Bacteroidetes* include Gram-negative bacteria; *Proteobacteria* consist of Gram-negative bacteria and includes a wide variety of well-studied pathogens. *Actinobacteria* are a group of Gram-positive bacteria.[6] The *Firmicutes* phylum is composed of *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*. *Bacteroidetes* phylum comprises four classes: *Bacteroidia, Flavobacteria*, *Sphingobacteria*, and *Cytophagia*.[7]

There has been a rapid rise in the use of antibiotics for various diseases among adults and children.[8] Infants, toddlers, and preschool children are usually treated with oral/intravenous antibiotics (e.g., penicillins, cephalosporins, and macrolides), due to their health conditions.[9] Antibiotics disrupt the composition of the gut microbiota,[10] affect the normal maturation of the microbiome, destabilizes it, and alter the basic physiological equilibria.[11] Studies on the effect of exposure to antibiotics show that the abundance and diversity of intestinal microbiota are affected irrespective of which type of antibiotics are used.[12] Early antibiotic exposure reduces microbiome composition and diversity with a marked reduction in *Bifidobacterium* and increases in *Proteobacterium* levels. Over time, when antibiotic treatment is stopped, the intestinal microbiota shows great resilience and returns to a composition like the original one, but it's not fully recovered in most cases.[13]

Several studies have demonstrated the impact of antibiotic usage on gut dysbiosis in adults, neonates, and infants. However, the short-term effect of antibiotics on gut dysbiosis in children is poorly understood.[6] Therefore, this study aims to investigate the effect of antibiotics on gut composition in children aged 3–5 years receiving antibiotics compared to children who did not receive antibiotics.

MATERIALS AND METHODS

Study design

A multi-centric cohort study was conducted at two sites in Pune, India. This study was conducted to investigate the impact of a short-term course of 5 days of antibiotic usage on gut microbiota and the differences in gut microbiota composition of participants receiving antibiotics compared to participants who did not.

Enrolled subjects

A total of 54 participants aged 3–5 years were screened and enrolled in this study, as the gut microbiota remains relatively stable within this age group. Participants were divided into two groups. In Group 1, there were 27 treatment-experienced participants who had an initial 1–2 days of IV antibiotics followed by oral antibiotics to treat infections for 5 days or more. In Group 2, there were 27 treatment-not-experienced participants who attended clinics for vaccinations, and non-infectious diseases were included in the study. Participants excluded from the study were those who had a medical history of any acute childhood illness in the past week, chronic illness, neonatal intensive care unit or pediatric intensive care unit admission, or history of drug intake in the past 3 months, including antibiotics, proton-pump inhibitors, and probiotics.

Sample collection

Stool samples from participants were collected on days 0 and 5 (± 1 day) of recruitment in outpatient and inpatient settings. After receiving antibiotics, stool samples were collected within 72 h of the completion of the antibiotic course. All the samples were collected in a 50 mL sterile falcon tube and stored at −80°C immediately. No preservative was used. All the samples were shipped in dry ice and sent for microbial analysis to determine differences in the gut composition of both groups.

Instruments/procedure

Microbial community analyses

Genomic deoxyribonucleic acid extraction

In this study, targeted metagenomics (amplicon) sequencing and bioinformatics services were used to analyze the stool samples. The total genomic Deoxyribonucleic acid (DNA) was extracted from 108 samples (54 participants) using 16Svedberg ribosomal ribonucleic acid (16S rRNA) gene amplicon

sequencing (Illumina MiSeq technology). To ensure quality and quantity prerequisites for targeted metagenomics (amplicon) sequencing Genomic deoxyribonucleic acid (gDNA) including 500 ng (minimum 20 ng/μL conc.) of community DNA, absorbance ratio (A260/280) of 1.8–2.0 and shipment of samples in cool pack were addressed.

Sequence processing and microbial community analysis

Quality trimming and adapter clipping of the Illumina sequences were done using Trimmomatic-0.38 paired end mode.[14] The trimmed and adapter free FASTQs were imported using quantitative insights into microbial ecology (QIIME) 2-2022.2 import tools.[15] Assembly of forward and reverse reads for each sample was carried out using vsearch join-pairs in qiime2.[16] Denoising the reads into amplicon sequence variants (ASVs) was done using deblur. Taxonomy was assigned to the ASVs with the SILVA138 database.[17] All possible contaminants were filtered out, including mitochondria, chloroplast, Eukaryota, and unassigned ASVs. A rooted phylogenetic tree was generated using FastTree and multiple alignment with fast fourier transform (MAFFT) and used in calculating phylogenetic diversity metrics. Data from QIIME 2 were analyzed and tested using various statistical packages, including "Phyloseq,"[18] "DESeq2,"[19] and "Vegan"[20] in R v.3.4.2. Alpha and beta diversity calculations were done using the tools for microbiome analysis in R.[21] Alpha diversity indices, including Shannon diversity and Chao, were calculated and analyzed using the Wilcoxon test to compare various sample types. Beta diversity was assessed using the Bray–Curtis distance matrix and depicted in a principal coordinate analysis (PCoA) plot. Detection of the differentially abundant phyla and genera across the samples was done using analysis of variance in GraphPad Prism ver. 9.0.

The differentially abundant phyla and genera were detected across the different sample categories by applying the Kruskal–Wallis H-test and Benjamini–Hochberg false discovery rate (FDR) correction using statistical analysis of metagenomic profiles (STAMP) (v2.1.3).

Metagenome-functional predictions and statistical analysis

Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) tool was used to analyze the metabolic potential of the microbial community[22] that predicts functional abundances based on marker gene sequences. The final output tables produced by PICRUSt2 represent the read depth per ASV multiplied by the predicted function abundances per ASV. The data were transformed to relative abundance before applying any statistical analysis. The relative abundance of identified pathways was compared across different zones and subzones.

Ethical aspects

The study was reviewed and approved by the Institutional Ethics Committee of KEM Hospital Research Center Pune (KEMHRC ID No. 2106). The ICMR's Ethical Guidelines for Biomedical and Health Research on human participants (2017) were followed. Written informed consent was obtained from the parents of each participant before recruitment.

Statistics

The Kruskal–Wallis H-test and Benjamini–Hochberg FDR correction were applied to determine the differentially abundant pathways across the zones using STAMP (v2.1.3). The predicted genes with a significant difference in their relative abundance ($P < 0.05$) were plotted in a heatmap matrix in the R package pheatmap (version 1.0.12).

RESULTS

A total of 54 participants aged 3–5 years were included in the study, with 27 participants in Group 1 (treatment-experienced) and 27 participants in Group 2 (treatment-not-experienced). A total of 108 stool samples were collected from

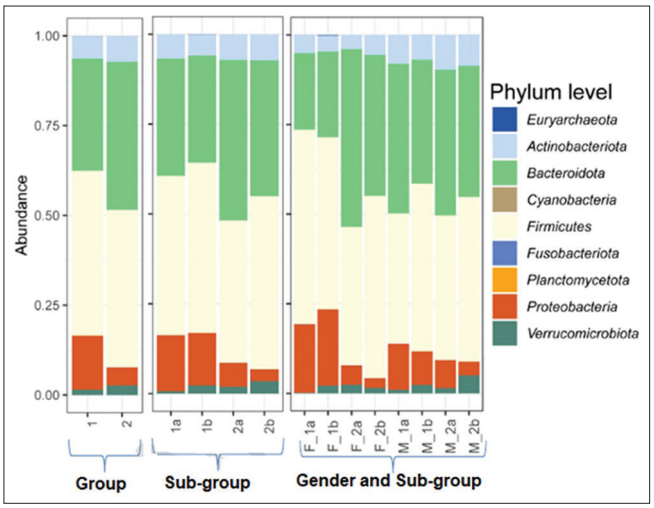


Figure 1: Relative abundance of phyla assigned to fecal microbiome of treatment-experienced and treatment-not-experienced participants on day 0 and day 5. Group 1 comprises treatment-experienced participants and group 2 comprises treatment-not-experienced participants. (1a) Subgroup treatment-experienced at day 0, (1b) subgroup experienced at day 5, (2a) subgroup treatment-not-experienced at day 0, and (2b) subgroup treatment-not-experienced at day 5. (F_1a) Treatment-experienced females at day 0, (F_1b) treatment-experienced females at day 5, (F_2a) treatment-not-experienced females at day 0, (F_2b) treatment-not-experienced females at day 5, (M_1a) treatment-experienced males at day 0, (M_1b) treatment-experienced males at day 5, (M_2a) treatment-not-experienced males at day 0 and (M_2b) treatment-not-experienced males at day 5 (M_2b).

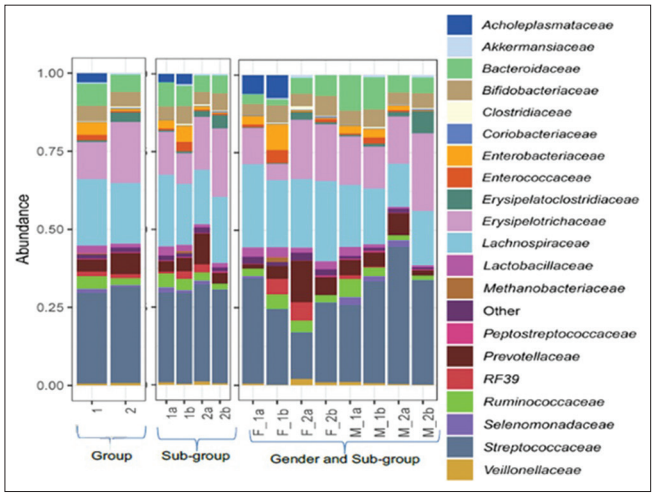


Figure 2: Relative abundance of family level assignments of the fecal microbiome of treatment-experienced and treatment -not-experienced female and male participants on zero and fifth day of sampling. Group 1 comprises treatment-experienced participants and group 2 comprises treatment-not-experienced participants. (1a) Subgroup treatment-experienced at day 0, (1b) subgroup treatment-experienced at day 5, (2a) subgroup treatment-not-experienced at day 0, and (2b) subgroup treatment-not-experienced at day 5. (F_1a) Treatment-experienced females at day 0, (F_1b) treatment-experienced females at day 5, (F_2a) treatment-not-experienced females at day 0, (F_2b) treatment-not-experienced females at day 5, (M_1a) treatment-experienced males at day 0, (M_1b) treatment-experienced males at day 5, (M_2a) treatment-not-experienced males at day 0, and (M_2b) treatment-not-experienced males at day 5.

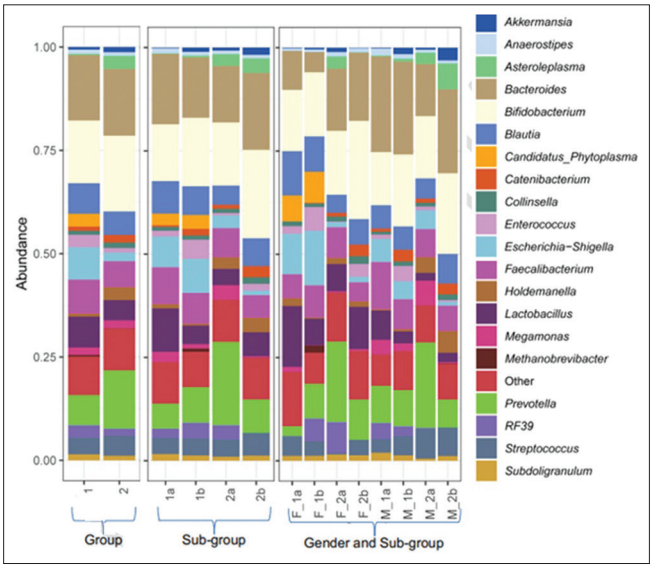


Figure 3: Relative abundance of genus level assignments of the fecal microbiome of treatment-experienced and treatment-not -experienced female and male participants on zero and fifth day of sampling. Group 1 comprises treatment-experienced participants and group 2 comprises treatment-not-experienced participants. (1a) Subgroup treatment-experienced at day 0, (1b) subgroup treatment-experienced at day 5, (2a) subgroup treatment-not-experienced at day 0, and (2b) subgroup treatment-not-experienced at day 5. (F_1a) Treatment-experienced females at day 0, (F_1b) treatment-experienced females at day 5, (F_2a) treatment-not-experienced females at day 0, treatment-not-experienced females at day 5 (F_2b), (M_1a) treatment-experienced males at day 0, (M_1b) treatment-experienced males at day 5, (M_2a) treatment-not-experienced males at day 0, and (M_2b) treatment-not-experienced males at day 5.

both groups on Day 0 (54 samples) and Day 5 (54 samples). All these samples were analyzed using 16S RNA gene amplicon sequencing.

Baseline demographics: In Group 1, 15 male and 12 female participants were included with a mean age of 46.4 months, whereas in Group 2, 16 male and 11 female participants were included with a mean age of 46.4 months.

The minimum and maximum gestational ages at birth between the two groups are between 37 and 40 weeks. About 40.7% of participants in Group I and 44.4% of participants in Group II had received breastfeeding. Participants having a normal diet in Group 1 and Group 2 are 33.3% and 30%, respectively. Normal vaginal delivery birth rates in Group 1 and II were 52% and 63%, respectively. In the 1st year of life, only 3.7% of Group 1 participants and 14.8% of Group 2 participants had received antibiotics. In the 2nd year, participants who received antibiotics in Group 1 and Group 2 were 48.1% and 40.7%, respectively. About 48.1% participants in Group 1 and 44.4% participants in Group 2 had received antibiotics at ages > 2 years.

The data have been categorized and analyzed at day 0 and day 5 for both males and females separately between the two groups.

At the phyla level, the mean relative abundance of *Bacteroidota* and *Firmicutes* in both breast-feed and non-breastfeed participants was $P = 0.1728$, $P = 0.1292$, $P = 0.1212$, and $P = 0.9663$, respectively, which was statistically less significant [Table 1]. A statistically significant difference was observed between the treatment-experienced and treatment-not-experienced groups on the mean relative abundance of *Bacteroidota* ($P = 0.0496$) and *Verrucomicrobiota* ($P = 0.0291$), while a less significant difference was seen in *Firmicutes* ($P = 0.2795$) [Table 2].

It was evident from the plot Figure 1 that there was a non-significant increase in the mean abundance of *Bacteroidota, Proteobacteria, Actinobacteria*, and *Verrucomicrobiota* in both groups from day 0 to day 5.

A mean relative increase in *Firmicutes* abundance was observed in both groups, more pronounced in Group 2 compared to Group 1.

At the phylum level, the mean relative abundance of *Firmicutes* was 1a < 1b, whereas that of *Bacteroidota* was 1b < 1a [Table 3] and sub categorization [Table 4].

Table 1: Impact of antibiotic treatment on mean relative abundance of gut microbiota by feed.

Feeding	Mean relative abundance (%)		
	Treatment-experienced (*n*=27) *n*, mean	Treatment-not-experienced (*n*=27) *n*, mean	*P*-values
	Breast-feed (*n*=23)		
Phylum			
Bacteroidota	11, 16.315	12, 21.699	0.1728
Firmicutes	11, 19.849	12, 15.197	0.1292
Family			
Enterobacteriaceae	11, 0.043	12, 0.018	0.401
Enterococcaceae	11, 0.018	12, 0.000	0.0068
Genus level			
Escherichia-Shigella	11, 5.773	12, 2.333	0.6228
Enterococcus	11, 1.818	12, 0.000	0.0068
	Non-breastfeed (*n*=31)		
Phylum			
Bacteroidota	16, 12.483	15, 16.317	0.1212
Firmicutes	16, 22.736	15, 21.710	0.9663
Family			
Enterobacteriaceae	16, 0.261	15, 0.059	0.0016
Enterococcaceae	16, 0.113	15, 0.027	0.0162
Genus level			
Escherichia-Shigella	16, 37.344	15, 7.100	0.0011
Enterococcus	16, 16.094	15, 3.767	0.0141

Table 2: Impact of antibiotic treatment on mean relative phyla level abundance of prokaryotic taxa.

Phylum	Mean relative abundance (%)		
	Treatment-experienced	Treatment-not-experienced	*P*-values
Bacteroidota	14.044	18.709	0.0496
Verrucomicrobiota	0.822	1.414	0.0291
Firmicutes	21.559	18.815	0.2795

Table 3: Impact of antibiotic treatment on the mean relative phyla level abundance of prokaryotic taxa among both groups.

Phylum	Mean relative abundance (%)				
	1a	1b	2a	2b	*P*-values
Bacteroidota	14.063	14.024	20.503	16.913	0.1743
Verrucomicrobiota	0.299	1.345	1.025	1.802	0.0722
Firmicutes	21.180	21.937	16.954	20.675	0.4562

1a: Sub-group treatment-experienced at day 0; 1b: Sub-group treatment-experienced at day 5; 2a: Sub-group treatment-not-experienced at day 0; 2b: Sub-group treatment-not-experienced at day 5

At the family level, the mean relative abundance of *Enterobacteriaceae* in breast-feed participants was $P = 0.401$, which was statistically not significant; whereas non-breastfeed participants, it was $P = 0.0016$, which was statistically significant. The mean relative abundance of *Enterococcaceae* in both breast-feed and non-breastfeed participants was $P = 0.0068$, and $P = 0.0162$, respectively, which was statistically significant [Table 1].

Figure 2 and Table 5 revealed an increase in the relative abundance of *Enterobacteriaceae*, *Enterococcaceae*, and *Peptostreptococcaceae* on day 5 over day 0 of sample collection in participants with antibiotic treatment. A significant decrease in the relative abundance of *Lactobacillaceae* was observed in Group 1 from day 0 to day 5.

A statistically significant ($P < 0.05$) increase in the relative abundance of *Enterococcaceae* and *Peptostreptococcaceae* is observed in Group 1 from day 0 to day 5. A similar increase was observed in the relative abundance in Group 2. However, the increase in *Enterococcaceae* was less in participants without antibiotics when compared to participants on antibiotics.

At the family level, the mean relative abundance of *Enterococcaceae* was 1a < 1b, whereas that of *Lactobacillaceae* was 1b < 1a [Table 6] and subcategorization [Table 7].

The mean relative abundance of *Escherichia-Shigella* at genus level in breast-feed participants was $P = 0.6228$, which was statistically non-significant; whereas non-breastfeed participants, it was $P = 0.0011$, which was statistically significant. The mean relative abundance of *Enterococcaceae* in both breast-feed and non-breastfeed participants was $P = 0.0068$, and $P = 0.0141$, respectively, which was statistically significant [Table 1].

Table 4: Impact of antibiotic treatment on mean relative phyla level abundance of prokaryotic taxa.

Phylum	F_1a	F_1b	F_2a	F_2b	M_1a	M_1b	M_2a	M_2b	*P*-values
Bacteroidota	8.137	10.563	22.087	16.583	18.804	16.793	19.236	17.177	0.0747
Verrucomicrobiota	0.086	1.338	1.467	0.478	0.469	1.351	0.672	2.861	0.2513
Firmicutes	27.529	23.815	16.442	20.626	16.102	20.435	17.364	20.715	0.4977

F_1a : Treatment-experienced females at day 0; F_1b: Treatment-experienced females at day 5; F_2a: Treatment-not-experienced females at day 0; F_2b: Treatment-not-experienced females at day 5; M_1a: Treatment-experienced males at day 0; M_1b: Treatment-experienced males at day 5; M_2a: Treatment-not-experienced males at day 0; M_2b: Treatment-not-experienced males at day 5

Table 5: Impact of antibiotic treatment on mean relative family level abundance of prokaryotic taxa.

Family	Mean relative abundance (%)		
	Treatment-experienced	Treatment-not-experienced	*P*-values
Enterobacteriaceae	0.172	0.041	0.028
Enterococcaceae	0.075	0.015	0.001
Lactobacillaceae	0.124	0.075	0.003
Prevotellaceae	0.162	0.360	0.035

Table 6: Impact of antibiotic treatment on the mean relative family level abundance of prokaryotic taxa among both groups.

Family	Mean relative abundance (%)				
	1a	1b	2a	2b	*P*-values
Enterococcaceae	0.0394	0.1097	0.0125	0.0171	0.001
Lactobacillaceae	0.1704	0.0778	0.0646	0.0854	0.033
Peptostreptococcaceae	0.0102	0.0113	0.0107	0.0287	0.022

1a: Sub-group treatment-experienced at day 0; 1b: Sub-group treatment-experienced at day 5; 2a: Sub-group treatment-not-experienced at day 0; 2b: Sub- group treatment-not-experienced at day 5

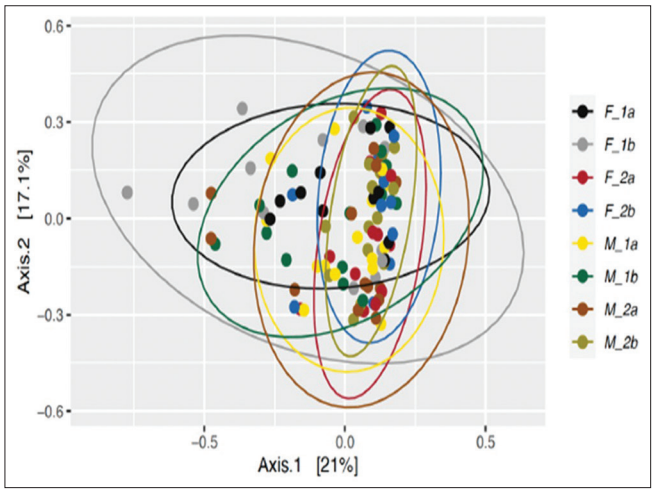


Figure 4: Principal coordinate analysis (PCoA) plots depicting beta-diversity calculated using the Bray-Curtis similarity distance among the prokaryotic communities in the fecal samples collected from the female participants at day 0 and day 5. (F_1a) Treatment-experienced females at day 0, (F_1b) treatment-experienced females at day 5, (F_2a) treatment -not-experienced females at day 0, (F_2b) treatment not experienced females at day 5, (M_1a) treatment-experienced males at day 0, (M_1b) treatment-experienced males at day 5, (M_2a) treatment-not-experienced males at day 0, and (M_2b) treatment-not-experienced males at day 5.

A significant decline was observed in the relative abundance of *Lactobacillus* from day 0 to day 5 in Group 1, while it increased from day 0 to day 5 in Group 2. The relative abundance of *Enterococcus* increased significantly from day 0 to day 5 in Group 1.

The relative abundance of *Bifidobacterium* increased from day 0 to day 5 in Group 1 and Group 2 participants. However, these changes are not statistically significant.

At the Genus level, the mean relative abundance of *Enterococcus* was 1a < 1b, whereas *Lactobacillus* was 1b < 1a [Figure 3 and Tables 8-10].

In Figure 4, beta-diversity indices revealed significant differences at group and subgroup levels regarding the bacterial counts. The biggest shift in the microbial community was observed in the antibiotic group for female participants from day 0 (black eclipse) to day 5 (gray eclipse); whereas a similar but smaller shift was observed for the male participant group with antibiotic treatment from day 0 (yellow eclipse) to day 5 (dark green eclipse). Among the antibiotic treatment groups for both male and female participants, the microbial communities were more heterogeneous after 5 days of antibiotic treatment. On the other hand, the microbial communities either remained similar or became more homogeneous on the 0 day and 5th day of sample collection for both participant groups without antibiotic treatment. However, a slight shift was noticed from the 0-day (red eclipse) to 5th day (blue eclipse) in samples collected from the female participants without antibiotic treatment. In the case of male participants without antibiotic treatment, the microbial communities become more homogenous on the 5th day (light green eclipse) than on the 0-day (brown eclipse).

The *Firmicutes*/Bacteroidetes (F/B) ratio is widely accepted to have an important influence on maintaining normal

Table 7: Impact of antibiotic treatment on mean relative family level abundance of prokaryotic taxa.

Family	F_1a	F_1b	F_2a	F_2b	M_1a	M_1b	M_2a	M_2b	*P*-values
Enterococcaceae	0.0531	0.1474	0.0026	0.0255	0.0283	0.0796	0.0204	0.0104	0.015
Lactobacillaceae	0.2026	0.1214	0.0995	0.1380	0.1446	0.0429	0.0367	0.0433	0.019
Peptostreptococcaceae	0.0063	0.0005	0.0073	0.0406	0.0133	0.0200	0.0133	0.0192	0.012

F_1a: Treatment-experienced females at day 0; F_1b: Treatment-experienced females at day 5; F_2a: Treatment-not-experienced females at day 0; F_2b: Treatment-not-experienced females at day 5; M_1a: Treatment-experienced males at day 0; M_1b: Treatment-experienced males at day 5; M_2a: Treatment-not-experienced males at day 0; M_2b: Treatment-not-experienced males at day 5

Table 8: Impact of antibiotic treatment on mean relative genus level abundance of prokaryotic taxa.

Genus	Mean relative abundance (%)		
	Treatment-experienced	Treatment-not-experienced	*P*-values
Ruminococcus torques group	0.0008	0.0000	0.0227
Dialister	0.0078	0.0137	0.0193
Eggerthella	0.0020	0.0002	0.0150
Enterococcus	0.0642	0.0131	0.0010
Escherichia-Shigella	0.1530	0.0311	0.0138
Lactobacillus	0.1236	0.0750	0.0033
Prevotella	0.0999	0.2368	0.0367
Ruminococcus	0.0021	0.0049	0.0051
Sellimonas	0.0014	0.0000	0.0425
Senegalimassilia	0.0003	0.0016	0.0457

Table 9: Mean relative genus level abundance of prokaryotic taxa among both groups.

Genus	Mean relative abundance (%)				
	1a	1b	2a	2b	*P*-values
Enterococcus	0.035	0.093	0.010	0.016	0.002
Lactobacillus	0.170	0.077	0.065	0.085	0.034
Ruminococcus	0.003	0.002	0.003	0.007	0.015

1a: Sub-group treatment-experienced at day 0; 1b: Sub-group treatment-experienced at day 5; 2a: Sub-group treatment-not-experienced at day 0; 2b: Sub- group treatment-not-experienced at day 5

intestinal homeostasis. An increased or decreased F/B ratio is considered as dysbiosis.[23] The presented study shows that the F/B ratios of Group 1 and Group 2 were 1.535 and 1.006, respectively [Tables 11-13].

DISCUSSION

Antibiotics are frequently used in children to treat common infections and diseases. However, little is known about the effects of antibiotics on the composition and load of the gut microbiota immediately after treatment.[2,6] The present study was, thus, undertaken to study how short-term antibiotic usage is associated with altered microbial abundance and diversity.

Furthermore, this study reasserts that there is an increasing need for global awareness and a detailed understanding of the relationship between antibiotic use and gut dysbiosis. The need of the hour is that healthcare practitioners consider the damage to the gut microbiome while prescribing antibiotics for children and limit their systematic use as they can reshape the microbiota in favor of resistant bacterial strains in the long term.

In the present study, *Firmicutes* and *Bacteroidota* represent a large majority of the prokaryotic communities in both groups that were exposed and not exposed to antibiotics for 5 days. This is in concordance with the findings of Wei *et al.* who observed that the most abundant phyla reported in the fecal samples of children were *Bacteroidetes* and *Firmicutes*, followed by the *Proteobacteria*, *Actinobacteria,* and *Verrucomicrobia*, 14 days after treatment with azithromycin in children aged 12–36 months.[8] On comparing groups with azithromycin and placebo, Parker *et al.* stated that the relative abundance of *Proteobacteria*, and *Verrucomicrobia* decreased on day 14. Study findings revealed a modest effect of azithromycin on the composition of the bacterial microbiota among 6–11-month-old infants.[24] The present study also showed a significant decrease in *Verrucomicrobia* mean relative abundance in the antibiotic-treated group but an increase in the *Proteobacteria* group, which could Possibly be due to the brief period of observation and short-term antibiotic usage in our study.

Ma *et al.* and Li *et al.* stated that breast milk is the main influence of gut microbiota, with differences observed among infants fed exclusively or with formula. *Firmicutes* on days 0 and 30 dominated the breast milk gut microbiota.[25,26] Similarly, the present study reported comparable results with gut microbiota composition. It was also observed in the present study that the mean relative abundance of *Bacteroidetes* significantly decreased, and there was a non-significant increase in the mean relative abundance of *Firmicutes* in both groups from day 0 to day 5. This was in contrast to the findings of Kwon *et al.*, who demonstrated a significant decrease in *Firmicutes* and *Bacteroidetes* phyla in the antibiotic group as compared to the control group in infants under 3 months of age.[2] *Firmicutes* count also decreased from 36% to 4% after 5 days of antibiotic treatment in a child with otitis media, as reported by Sturød *et al.*[27]

The present study demonstrated a significant increase in the *Escherichia-Shigella* groups at the genus level in the antibiotic

Table 10: Impact of antibiotic treatment on mean relative genus level abundance of prokaryotic taxa.

Genus	F_1a	F_1b	F_2a	F_2b	M_1a	M_1b	M_2a	M_2b	*P*-values
(*Ruminococcus*)_torques_group	0.003	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.013
Eggerthella	0.001	0.005	0.000	0.000	0.001	0.002	0.000	0.001	0.013
Enterococcus	0.049	0.122	0.002	0.024	0.024	0.070	0.018	0.009	0.025
Lactobacillus	0.202	0.120	0.099	0.138	0.145	0.043	0.037	0.043	0.020
Olsenella	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.024

F_1a: Treatment-experienced females at day 0; F_1b: Treatment-experienced females at day 5; F_2a: Treatment-not-experienced females at day 0; F_2b: Treatment-not-experienced females at day 5; M_1a: Treatment-experienced males at day 0; M_1b: Treatment-experienced males at day 5; M_2a: Treatment-not-experienced males at day 0; M_2b: Treatment-not-experienced males at day 5

Table 11: F/B ratio of treatment experienced and treatment not experienced groups.

Phylum	Treatment-experienced	Treatment-not-experienced
Firmicutes	21.559	18.815
Bacteroidota	14.044	18.709
F/B ratio	1.535	1.006

F/B: *Firmicutes/Bacteroidetes*

Table 12: F/B ratio of treatment experienced and treatment not experienced groups at day 0 and day 5.

Ratio	Subgroup			
	1a	1b	2a	2b
F/B ratio	1.506	1.564	0.827	1.222

F/B: *Firmicutes/Bacteroidetes,* 1a: sub-group treatment-experienced at day 0;1b: sub-group treatment-experienced at day 5; 2a: sub-group treatment-not-experienced at day 0; 2b: sub-group treatment-not-experienced at day 5.

Table 13: F/B ratio of treatment experienced and treatment not experienced groups for females and males at day 0 and day 5.

F/B ratio	1a	1b	2a	2b
Female	3.382	2.254	0.744	1.244
Male	0.856	1.217	0.903	1.206

F/B: *Firmicutes/Bacteroidetes,* 1a: sub-group treatment-experienced at day 0; 1b: sub-group treatment-experienced at day 5; 2a: sub-group treatment-not-experienced at day 0; 2b: sub-group treatment-not-experienced at day 5

group as compared to the non-antibiotic group. This was in concordance with the study of Kwon *et al.*, who reported a significant increase in the *Escherichia*/*Shigella*, and *Bifidobacterium* groups at the genus level in the antibiotic group as compared to the control group.[2] Wei *et al.* also found that the *Bifidobacterium* count significantly reduced in the azithromycin group at day 14 of fecal sample analysis.[8] While Mangin *et al.* found no significant differences in total *Bifidobacteria* concentrations after amoxicillin treatment for 7 days, the average number of *Bifidobacterium* species per microbiota was significantly lower for treated infants compared to the healthy group at day 7.[28]

Alpha diversity indices in the present study suggested no significant differences between the two groups at baseline or post-treatment [Supplementary Figure 1]. This was unlike the study of Oldenburg *et al.*, who found non-significant differences at baseline and significant differences in Simpson's (0.003) and Shannon's (0.0001) α-diversity indices in all four treatment arms on the 5th day post-treatment.[10] Furthermore, in a study conducted by Kwon *et al.*, similarly, significant differences in Chao1 (0.033) and Shannon index (0.009) between the control and the antibiotic groups at 4 weeks of sample collection were observed.[2] Similar to our study, Doan *et al.* found that alpha diversity indices at baseline were non-significant across the two groups, but analysis of stool samples after 5 days of antibiotic treatment revealed significant changes in Inverse Simpsons' α-diversity indices, with the antibiotic-treated group showing decreased microbial count.[29] Wei *et al.* also demonstrated that the Shannon diversity index showed statistically lower results for the azithromycin group as compared to the placebo on 14 days of antibiotic use.[8]

In the present study, the β-diversity indices showed that the microbial communities in the antibiotic-treated group were more heterogeneous on day 5 as compared to the non-antibiotic treated group, which showed a more homogenous composition on both days. This finding harmonizes with the study of Bokulich *et al.*, who in their analysis, found a significant relation in the β-diversity index of stool samples of children collected over 2 years. In their study, antibiotic exposure was associated with deficits in *Clostridiales* and *Ruminococcus* from 3 to 9 months of life but with no consistent changes in other taxa.[30] On the other hand, Doan *et al.* reported that β-diversity indices did not show any significance in the azithromycin versus placebo group 5 days post-treatment.[29]

The present study also revealed that a higher abundance of *Erysipelatoclostridium*, *Clostridium* species, *Ruminococcus*, and *Eschirichia-Shigella* was seen on day 5 of antibiotic treatment [Supplementary Figure 2]. This is in contrast to a study by Abeles *et al.* (2016), who observed depletion of the *Erysipelotrichaceae*, *Veillonellaceae*, and *Clostridiales* in the

gut flora of children following a 3 or 7 day antibiotic course.[31] Doan *et al.* stated in their study that *Faecalibacterium*, *Blautia*, *Bifidobacterium*, *Succinivibrio*, *Ruminococcus*, *Roseburia*, *Escherichia*, and *Clostridium*, account for 61% (higher abundance) of the filtered reads on days 0 and 5 for Antibiotic-treated group.[29]

The present study has shown that the relative abundance of *Enterococcus*, *Lactobacillus*, *Sellimonas*, and *Eggerthella* was significantly higher in participants receiving antibiotic treatment. On the contrary, the relative abundance of *Prevotella*, *Dialister*, and *Senegalimassilia* was lower in participants with antibiotic treatment in comparison to participants without antibiotic treatment. Prediction of the functions of prokaryotic communities thereby revealed a higher abundance of genes associated with antibiotic resistance in the samples from the participants with antibiotic treatment in comparison to the non-antibiotic group.

In their study, Panda *et al.* (2014) reported that fluoroquinolones and b-lactams significantly decreased microbial diversity by 25% and reduced the core phylogenetic microbiota from 29 to 12 taxa. However, at the phylum level, these antibiotics increased the *Bacteroidetes*/*Firmicutes* ratio (B/F ratio) (P = 0.0007, FDR = 0.002).[6] In contrast to this, the present study demonstrates that antibiotics increased the phylum *Firmicutes* from day 0 to day 5, while *Bacteroidota* decreased from day 0 to day 5, decreasing the B/F ratio. In our study, the F/B ratio of the antibiotic group was 1.535. In subgroup analysis, the F/B ratio of group 1 at day 0 and day 5 was 1.506 and 1.564, respectively.

The differences in these findings obtained in our study and previous studies could possibly be attributed to the age of the study participants, lifestyle-associated factors including diet, physical activity, food additives and contaminants, antibiotic consumption, physical activity, the study setting (different geographical areas have been shown to have different microbial compositions in the intestine), the study duration, and the time since the antibiotic exposure.

This study has few limitations considering the short-term duration of post-antibiotic exposure observation and the inclusion of participants from the same region. Furthermore, this study did not give importance to the class and type of antibiotics used by the study participants.

CONCLUSIONS

Several studies have demonstrated that the gut microbiome is sensitive to antibiotic treatment. However, there has been little to no information available on the short-term usage of antibiotics in children aged 3–5 years. This is the first Indian study conducted on children to determine the differences in the gut composition of participants receiving antibiotics compared to participants who did not receive antibiotics.

In our study, it was observed that there was a significant decrease in the gut flora of study participants who belonged to the treatment-experienced group from day 0 to day 5 at the phyla, family, and genus level, whereas in the treatment-not-experienced group, there were no significant changes in the gut flora from day 0 to day 5. Future studies involving specific classes of antibiotics to study their effects on the gut microbiome are warranted.

Acknowledgments

The authors would like to thank the study participants and facilities for their cooperation and contribution to the completion of this study. The authors sincerely acknowledge the support and consultation from Microgenomics and Technology Solutions, Mandi (H.P) and Techsol Lifesciences, Hyderabad, Telangana. This study was funded by Dr. Reddy's Laboratories Ltd., Hyderabad, India.

Ethical approval

The author(s) declare that they have taken the ethical approval from IEC (KEMHRC ID No. 2106).

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

Financial support and sponsorship

This research was funded by Dr. Reddy's Laboratories Ltd., but no financial relationship or activity was with any of the primary investigators.

Conflicts of interest

All authors declare the following: Payment/services info: This research was funded by Dr Reddy's Laboratories Ltd., Financial relationships: Neha, Devesh Kumar Joshi, Krishna Chaitanya Veligandla, Rahul Rathod, Bhavesh Kotak declare(s) employment from Dr. Reddy's Laboratories Ltd. The authors, Neha and Devesh Kumar Joshi are serving as the Medical Advisors at Dr. Reddy's Laboratories Ltd. Hyderabad. Similarly, the authors, Krishna Chaitanya Veligandla, Rahul Rathod, and Bhavesh Kotak are also working as Medical Cluster Head, Head Ideation and Clinical Research, and Head Medical Affairs, respectively, at Medical Affairs Department, Dr. Reddy's Laboratories Ltd. Hyderabad, India. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

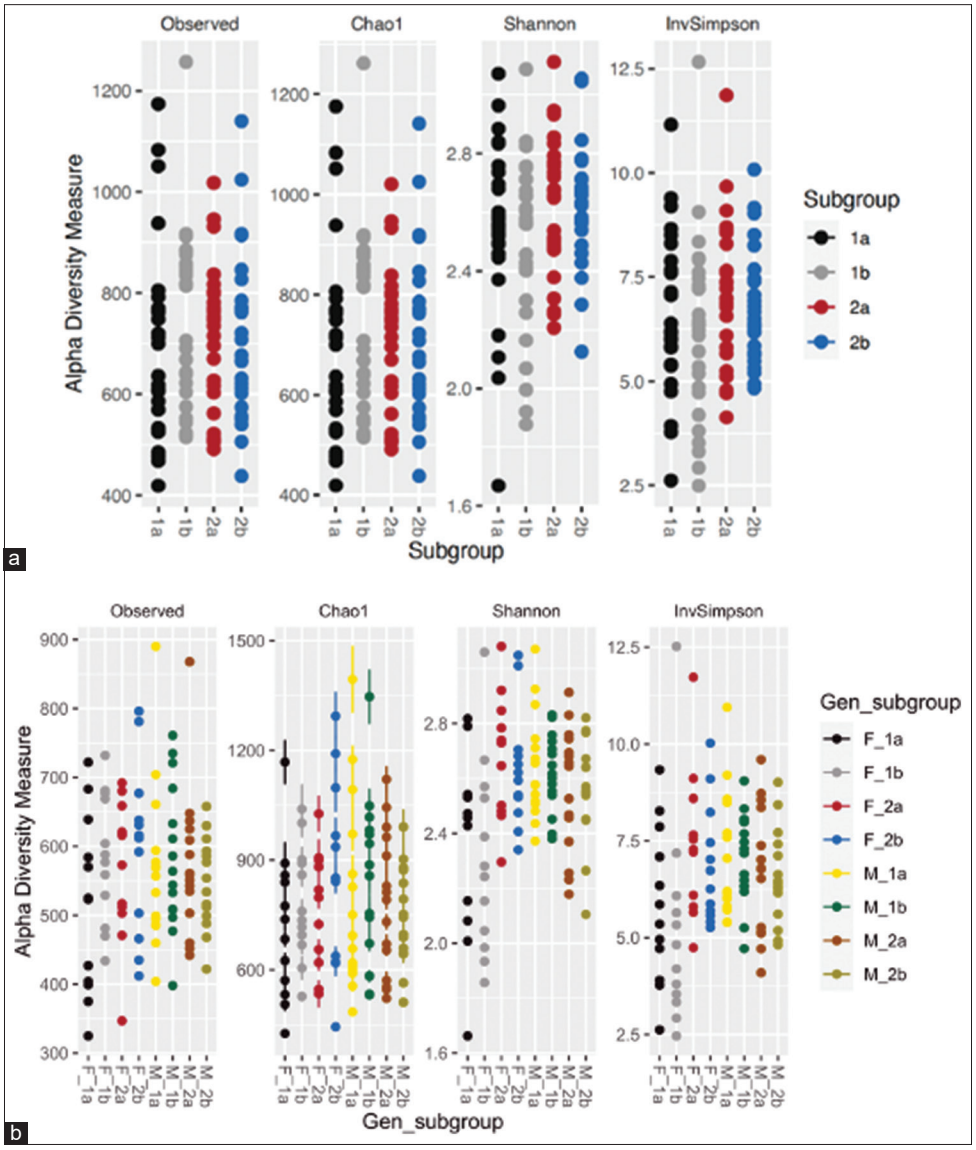
The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

REFERENCES

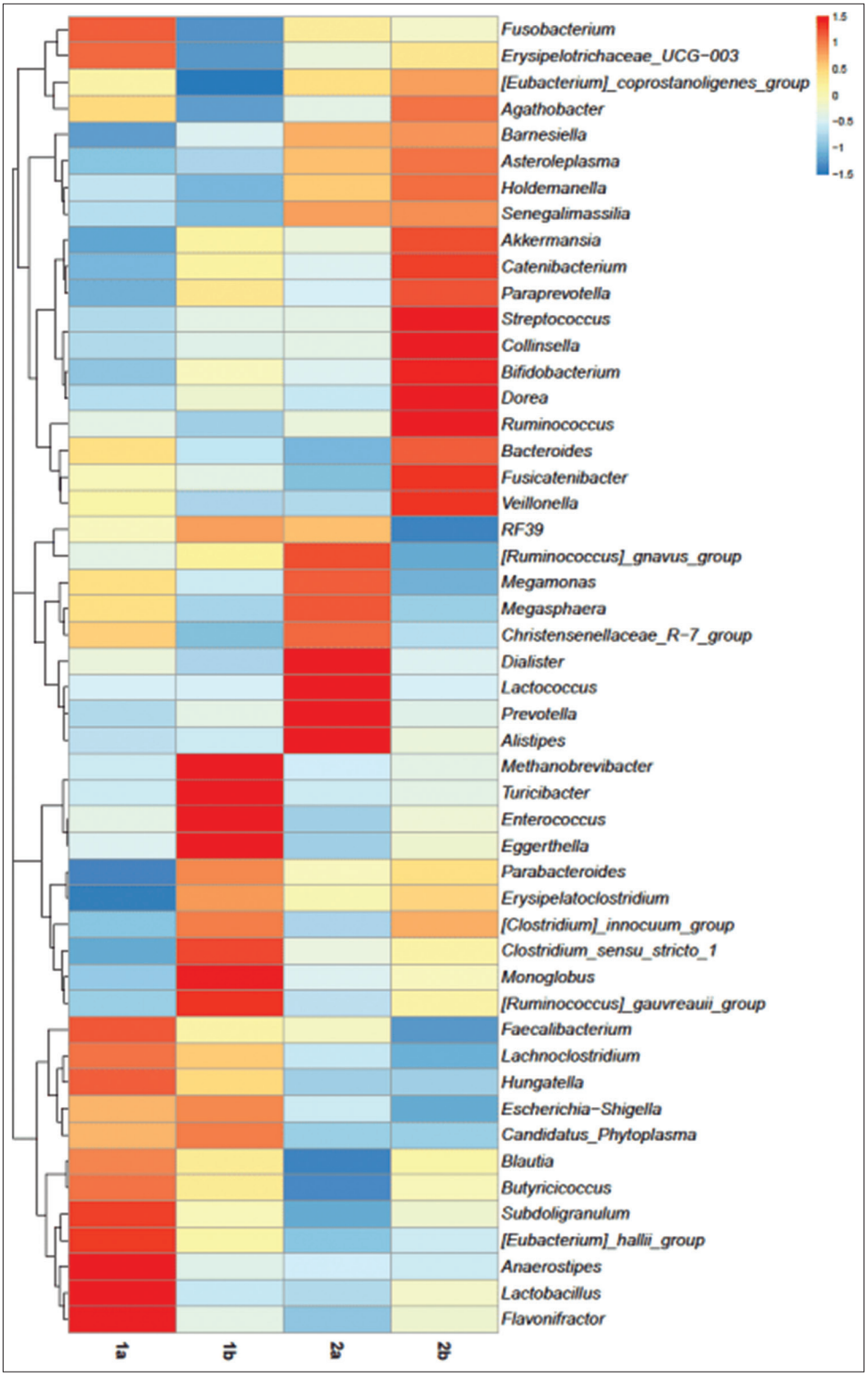
1. Imoto N, Kano C, Aoyagi Y, Morita H, Amanuma F, Maruyama H, *et al.* Administration of β-lactam antibiotics and delivery method correlate with intestinal abundances of Bifidobacteria and *Bacteroides* in early infancy, in Japan. Sci Rep 2021;11:6231.
2. Kwon Y, Cho YS, Lee YM, Kim SJ, Bae J, Jeong SJ. Changes to gut microbiota following systemic antibiotic administration in infants. Antibiotics (Basel) 2022;11:470.
3. Kumbhare SV, Patangia DV, Patil RH, Shouche YS, Patil NP. Factors influencing the gut microbiome in children: From infancy to childhood. J Biosci 2019;44:49.
4. Ramirez J, Guarner F, Bustos Fernandez L, Maruy A, Sdepanian VL, Cohen H. Antibiotics as major disruptors of gut microbiota. Front Cell Infect Microbiol 2020;10:572912.
5. Ohland CL, Jobin C. Microbial activities and intestinal homeostasis: A delicate balance between health and disease. Cell Mol Gastroenterol Hepatol 2015;1:28-40.
6. Panda S, El Khader I, Casellas F, López Vivancos J, García Cors M, Santiago A, *et al.* Short-term effect of antibiotics on human gut microbiota. PLoS One 2014;9:e95476.
7. Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G. Environmental and gut bacteroidetes: The food connection. Front Microbiol 2011;2:93.
8. Wei S, Mortensen MS, Stokholm J, Brejnrod AD, Thorsen J, Rasmussen MA, *et al.* Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: A double-blind, randomized, placebo-controlled trial. EBioMedicine 2018;38:265-72.
9. Patangia DV, Anthony Ryan C, Dempsey E, Paul Ross R, Stanton C. Impact of antibiotics on the human microbiome and consequences for host health. Microbiologyopen 2022;11:e1260.
10. Oldenburg CE, Sié A, Coulibaly B, Ouermi L, Dah C, Tapsoba C, *et al.* Effect of commonly used pediatric antibiotics on gut microbial diversity in preschool children in Burkina Faso: A randomized clinical trial. Open Forum Infect Dis 2018;5:ofy289.
11. McDonnell L, Gilkes A, Ashworth M, Rowland V, Harries TH, Armstrong D, *et al.* Association between antibiotics and gut microbiome dysbiosis in children: Systematic review and meta-analysis. Gut Microbes 2021;13:1-18.
12. Yoon MY, Yoon SS. Disruption of the gut ecosystem by antibiotics. Yonsei Med J 2018;59:4-12.
13. Francino MP. Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. Front Microbiol 2016;6:1543.
14. Bolger AM, Lohse M, Usadel B. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 2014;30:2114-20.
15. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 2019;37:852-7.
16. Rognes T, Flouri T, Nichols B, Quince C, Mahé F. VSEARCH: A versatile open source tool for metagenomics. PeerJ 2016;4:e2584.
17. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al.* The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590-6.
18. McMurdie PJ, Holmes S. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8:e61217.
19. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
20. Dixon P. VEGAN, a package of R functions for community ecology. J Veg Sci 2003;14:927-30.
21. Lahti L, Shetty S. Tools for microbiome analysis in R. Version; 2017. Available from: https://microbiome.github.io/tutorials/Alphadiversity.html
22. Douglas GM, Maffei VJ, Zaneveld JR, Yurgel SN, Brown JR, Taylor CM, *et al.* PICRUSt2 for prediction of metagenome functions. Nat Biotechnol 2020;38:685-8.
23. Stojanov S, Berlec A, Štrukelj B. The influence of probiotics on the firmicutes/bacteroidetes ratio in the treatment of obesity and inflammatory bowel disease. Microorganisms 2020;8:1715.
24. Parker EPK, Praharaj I, John J, Kaliappan SP, Kampmann B, Kang G, *et al.* Changes in the intestinal microbiota following the administration of azithromycin in a randomized placebo-controlled trial among infants in south India. Sci Rep 2017;7:9168.
25. Ma J, Li Z, Zhang W, Zhang C, Zhang Y, Mei H, *et al.* Comparison of gut microbiota in exclusively breast-fed and formula-fed babies: A study of 91 term infants. Sci Rep 2020;10:15792.
26. Li Y, Ren L, Wang Y, Li J, Zhou Q, Peng C, *et al.* The effect of breast milk microbiota on the composition of infant gut microbiota: A cohort study. Nutrients 2022;14:5397.
27. Sturød K, Dhariwal A, Dahle UR, Vestrheim DF, Petersen FC. Impact of narrow-spectrum penicillin V on the oral and faecal resistome in a young child treated for otitis media. J Glob Antimicrob Resist 2020;20:290-7.
28. Mangin I, Suau A, Gotteland M, Brunser O, Pochart P. Amoxicillin treatment modifies the composition of *Bifidobacterium* species in infant intestinal microbiota. Anaerobe 2010;16:433-8.
29. Doan T, Arzika AM, Ray KJ, Cotter SY, Kim J, Maliki R, *et al.* Gut microbial diversity in antibiotic-naive children after systemic antibiotic exposure: A randomized controlled trial. Clin Infect Dis 2017;64:1147-53.
30. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, *et al.* Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med 2016;8:343ra82.
31. Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, *et al.* Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome 2016;4:39.

How to cite this article: Neha, Bavdekar A, Kawade A, Veligandla KC, Joshi DK, Rathod R, *et al.* Short term antibiotic effects on gut microbiome in Indian preschoolers: A 16S rRNA analysis. J Lab Physicians. 2024;16:7-18. doi: 10.25259/JLP_6_2023

SUPPLEMENTARY FIGURES



Supplementary Figure 1: (a and b) Prokaryotic communities in fecal microbiome of subgroups. (1a) Subgroup treatment-experienced at day 0, (1b) subgroup treatment-experienced at day 5, (2a) subgroup treatment-not-experienced at day 0, and (2b) subgroup treatment-not-experienced at day 5. Gen_subgroup: (F_1a) Treatment-experienced females at day 0, (F_1b) treatment-experienced females at day 5, (F_2a) treatment-not-experienced females at day 0, (F_2b) treatment-not-experienced females at day 5, (M_1a) treatment-experienced males at day 0, (M_1b) treatment-experienced males at day 5, (M_2a) treatment-not-experienced males at day 0, and (M_2b) treatment-not-experienced males at day 5.



Supplementary Figure 2: Heatmap depicting the abundance of prokaryotic genera of subgroups on day 0 and day 5. (1a) Subgroup treatment-experienced at day 0, (1b) subgroup treatment-experienced at day 5, (2a) subgroup treatment-not-experienced at day 0, and subgroup (2b) treatment-not-experienced at day 5.