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Original Article

GUT MICROBIOTA DYSBIOSIS IN PEDIATRIC DIARRHEA: INSIGHTS FROM A PILOT STUDY IN DUHOK, KURDISTAN REGION OF IRAQ

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ABSTRACT

Diarrhea in children remains a significant global health burden and is closely linked to gut microbiota dysbiosis. However, data from Middle Eastern populations, particularly Iraq, remain limited. This study aims to characterize the change of gut microbiota associated with diarrhea in Iraqi children and to identify the potential of microbial imbalance by comparison with healthy controls. Stool samples were collected from 10 children (6 with diarrhea, 4 healthy controls) at Hevi Pediatric Teaching Hospital, Duhok, Iraq. Bacterial DNA was extracted and analyzed using 16S rRNA amplicon sequencing (V3–V4 region, Illumina NovaSeq). Sequence data were processed with QIIME2 software to assess taxonomic structure, α- and β-diversity. Comparisons between groups were performed using the Wilcoxon signed-rank test and the Metacoder Tree test. In this study, 241 bacterial taxa belonging to 11 phyla were identified. An enrichment in *Proteobacteria* and *Bacteroidota* was observed in cases of diarrhea, while the abundance of *Firmicutes*, alongside *Ruminococcaceae, Lachnospiraceae,* and *Akkermansia muciniphila*, was reduced. Notably, beneficial SCFA-producing genera, including *Blautia*, *Faecalibacterium*, and *Bifidobacterium*, were significantly reduced, while *Enterobacteriaceae* and *Enterococcaceae* were increased. Elevated ratios of *Bacteroidota/Firmicutes* and *Proteobacteria/Firmicutes* ($p < 0.05$) were also noted. Although no statistically significant differences were observed for α-diversity, β-diversity confirmed distinct clustering of both groups of diarrheal versus healthy microbiota. Diarrhea in Iraqi children was characterized by a shift of microbiome and a marked overgrowth of *Proteobacteria*, along with depletion of *Firmicutes* and loss of SCFA-producing genera, consistent with indicators of global microbial imbalance. However, the elevated *Bacteroidota* suggests region-specific indicators. These population-level variations may serve as biomarkers of imbalance. Further, larger, longitudinal, multi-omic studies are required to validate these findings.

KEYWORDS: Gut microbiome, Illumina NovaSeq, Pediatric Diarrhea, QIIME2.

1. INTRODUCTION

The human gut microbiota consists of a complex community of microorganisms, including bacteria, viruses, and fungi. These microorganisms play an important role in maintaining intestinal health, balancing metabolism, and regulating the immune system (Li *et al*., 2021). Studies have shown that the gut microbiota in healthy individuals is characterized by high diversity and a predominance of beneficial commensal bacteria, which not only help maintain gut homeostasis but also prevent pathogen colonization (Iancu *et al*., 2023; Li *et al*., 2021; Tesfaw *et al*., 2024). However, the gut microbiota undergoes significant changes in patients experiencing diarrhea, commonly referred to as dysbiosis. It has been confirmed that this dysbiosis is characterized by a depletion of beneficial commensals, such as *Bifidobacterium* spp. and *Lactobacillus* spp. alongside an overgrowth of pathogenic bacteria such as *Escherichia*

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spp. and *Campylobacter* spp. (Du *et al*., 2023; The & Le, 2022; Tesfaw *et al*., 2024).

The disruption of the gut microbiota during diarrhea not only impairs the fundamental structural and functional integrity of the gut mucosa but also weakens immunity, increasing the host's susceptibility to further inflammation and infections (Iancu *et al*., 2023; Li *et al*., 2021). In spite of most diarrheal cases being short-lived, the resulting microbial imbalance can have lasting effects, particularly in high-risk groups such as the elderly and children (The & Le, 2022). Almost all studies have shown that restoring a healthy gut microbiota via dietary interventions, probiotics, and, very recently, fecal microbiota transplantation has shown promise in alleviating diarrheal symptoms and re-establishing microbial balance (Du *et al*., 2023; Iancu *et al*., 2023; Li *et al*., 2021). Comparative studies indicate that individuals with diarrhea have lower gut microbial diversity, greater abundance of pathogenic bacteria, and fewer beneficial microbes than healthy individuals. Du and his colleagues mentioned that this highlights the crucial role of gut microbiota in both the pathogenesis and resolution of diarrheal diseases (Du *et al*., 2023).

Recently, studies on the human gut microbiome and its role in health and disease have opened new avenues for scientific inquiry, providing critical insights into the structure and function of this highly complex ecosystem (Iancu *et al*., 2023; Li *et al*., 2021). Advances in bioinformatics and Next-Generation sequencing technologies now allow for comprehensive characterization of microbial communities and prediction of their functional capacities (Tesfaw *et al*., 2024; The & Le, 2022). However, despite these global advancements, studies on gut microbiome dynamics remain limited within Iraqi academic and clinical settings. To date, only a few investigations have been conducted in adults (Hama-Ali & Hasan, 2023; Kadhim *et al*., 2025), alongside several reports on the sporadic isolation of microbial species causing diarrhea in children (Ali, 2025; Hasan *et al*., 2020). To the best of our knowledge, this is the first study aimed to investigate taxonomic variations in gut microbiome composition between diarrheal and healthy pediatric populations in Iraq.

2. MATERIALS AND METHODS

Stool samples and DNA extraction:

A total of 10 stool samples (6 from children with diarrhea, 4 from healthy children) were randomly collected at Hevi Pediatric Teaching Hospital in Duhok, Iraq, in October 2024, with ages ranging from 4 to 6 years. The samples were immediately transported in a cool box containing dry ice to the microbiology research laboratory in the Department of Biology, College of Science, University of Duhok, for direct DNA extraction.

All samples were preserved using a stool transport and recovery buffer (S.T.A.R., Roche, Mannheim, Germany), kept on ice, and transferred to the Microbiology Laboratory prior to Bacterial DNA extraction. DNA was isolated using a High Pure PCR Template Preparation kit (Roche, Germany) according to the manufacturer's protocol. DNA concentration was achieved using a Nanodrop DeNOVIX (Wilmington, USA) with values ranging from 120 to 256 ng/µL. The purity ratios of both 260/280 and 230/280 were between 1.8 and 2.2. Then, this DNA was immediately stored at -20 °C to preserve its integrity and ensure a high-quality yield for downstream microbiome studies.

16S rRNA amplicons sequencing and Bioinformatic and abundance analysis:

The extracted bacterial DNA was then sent to BMKGENE Biomarker Technologies (Hong Kong) Company Limited, China (www.bmkgene.com) for Next-Generation Sequencing (NGS) using 16S rRNA Amplicon Sequencing on an Illumina NovaSeq platform (PE250-Seq) with 2 × 250 base paired-end reads. Universal primers targeting the V3-V4 hypervariable regions of the 16S rRNA gene were used to amplify the gene. The raw sequence FASTQ files were processed using the QIIME2 pipeline, following previously described studies (Biswas *et al*., 2021; Ibrahim *et al*., 2020, 2021; Kadhim *et al*., 2025). DADA2 was applied for denoising, and trimming parameters were selected on the per-base quality score, low-quality bases, and primer remnants at the start were removed using an 18 bp trim, and reads 240 bp were truncated (Callahan *et al*., 2016). The bacterial taxonomic classification was performed using the sepp-refs-gg-13-8 reference from the Greenegene database, targeting the gg-13-8-99-515-806-nb-classifier.

Statistical analysis:

Taxa read was normalized at all levels for each sample to generate relative abundance prior to analysis. The taxa's relative abundance is presented as a mean for each group and visualized by stacked bars. The non-parametric Wilcoxon Rank Sum test of two groups' comparison was used to compare the ratio of two phyla between the two groups. In addition, the taxonomic diversity (α and β metrics), Dendrogram, and Heat tree analyses by Metacoder Tree were performed using several MicrobiomeAnalyst. Results with p values of <0.05 were considered statistically significant.

3. RESULTS

Basic statistics of sequence reads:

The total number of reads and clusters of sequences from fecal specimens of 10 children (6 with diarrhea and 4 controls) was 1,599,551. The analysis was performed using QIIME II software. After the reads overlapped to merge paired reads for good quality reads in each sample, these sequences were assigned, filtered to remove uncombinable read pairs, and the non-chimeric combined reads were 572937 for diarrhea and 532759 for healthy samples (Table 1). These reads were then used for final taxa analysis.

Table 1: Summary statistics of quality-controlled sequencing reads for both groups.

Sample ID	input	filtered	percentage of input passed filter	denoised	Merged	percentage of input merged	non-chimeric	percentage of input non-chimeric
Diarrhea1	159913	144372	90.3	142913	136902	85.6	115577	72.3
Diarrhea2	160011	145025	90.6	143114	135695	84.8	111406	69.6
Diarrhea3	159854	145601	91.1	143141	135134	84.5	97152	60.8
Diarrhea4	160299	145357	90.7	144353	139787	87.2	121148	75.6
Diarrhea5	159738	143227	89.7	142368	138718	86.8	127654	79.9
Diarrhea6	799815	723582	90.5	715889	686236	85.8	572937	71.6
Healthy1	159914	144408	90.3	144091	142317	89	134216	83.9
Healthy2	159866	145972	91.3	145060	141786	88.7	128573	80.4
Healthy3	159900	144607	90.4	144414	143694	89.9	141124	88.3
Healthy4	160453	146067	91	144835	141117	88	128846	80.3
Total/%	2239763	2028218	90.59	2010178	1941386	87.03	1678633	76.27

Taxa relative abundance and phylogenetic composition:

From these total reads, 241 bacterial taxa across 11 phyla, 37 families, and 62 genera were identified in both diarrhea and healthy children. The composition and relative abundance differed between the two groups at all three taxonomic levels.

The analysis of the gut microbiota at the phylum level is shown in Figure 1. In the diarrheal samples, the relative abundance of certain phyla was notably higher than in the healthy controls (Figure 1a). The diarrhea samples exhibited a higher proportion of *Bacteroidota* (14.57%), *Proteobacteria* (12.26%), and *Actinobacteriota* (1.2%) than in healthy stools (0.50%, 2.58%, and 0.04%, respectively). Regarding the *Firmicutes* subgroups, notable differences in distribution patterns were observed: *Firmicutes* A showed lower abundance (14.11%) in diarrhea than in healthy samples (25.22%), while *Firmicutes* D showed greater abundance (32.35%) in healthy samples and 20.84% in diarrhea. Overall, the proportion of *Firmicutes* appeared reduced in diarrhea. The phylum *Actinobacteriota* maintained a similar relative abundance of approximately 35% in both groups, while the phylum *Verrucomicrobiota* was highest (1.85%) in healthy samples compared to 0.0% in diarrhea.

The relative abundance of gut microbes was assessed and compared between the diarrhea and healthy groups (Figure 1b). Notably, significant differences were observed in the ratios of both *Bacteroidota* and *Proteobacteria* to *Firmicutes* D (p=0.016 and p=0.019, respectively) and *Bacteroidota* to *Firmicutes* A (p=0.009). In these three ratios comparisons, the Diarrhea group exhibited a much higher ratio and greater variability compared to a tightly clustered group at low levels in the healthy group.

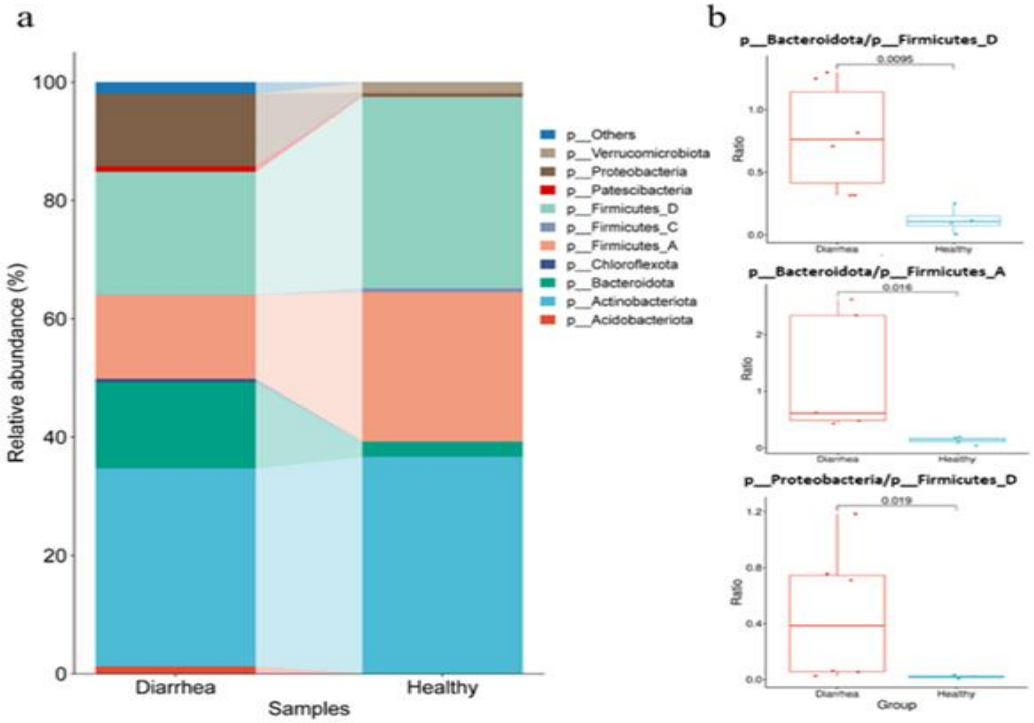

Figure 1: Gut microbiota comparison at the phylum level between Diarrhea and Healthy children.

Stacked bar plots show the mean relative abundance of bacterial phyla in the diarrhea (red) and healthy (blue) groups (a). Box plot comparing the selected phylum ratio between groups. A statistically significant difference (p-value <0.05) was indicated (b).

The relative abundance of various bacterial families across individual samples is shown in Figure 2a. The data revealed a more diverse and fragmented microbial community in the Diarrhea group, while the Healthy group exhibited fewer but more dominant bacterial families. For example, higher relative abundances of beneficial families such as *Bifidobacteriaceae* (28.86%), *Streptococcaceae* (22.10%), and *Lachnospiraceae* (19.34%), and followed by *Coprobacillaceae* (8.44%) and *Ruminococcaceae* (4.17%) observed in the Healthy group, while these same families were markedly reduced in the Diarrhea group, with respective abundances of 20.17%, 1.69%, 13.02%, 3.11%, and 0.37%. On the other hand, a broader spectrum of less dominant families was observed in the Diarrhea group, indicating gut microbial dysbiosis. These families are *Flavobacteriaceae* (5.67%), *Planococcaceae* (3.11%), *Alkalibacillaceae* (2.68%), *Lactobacillaceae* (2.29%), *Enterobacteriaceae* (1.65%), and *Xanthbacteraceae* (3.14%), which showed increased variability. Additionally, roughly 14.20% of other families (less than 0.5%) were also observed in the Diarrhea group.

The comparative relative abundance of genera reveals a distinct difference between the Diarrhea and Healthy groups (Figure 2b). Similar to families, there was a more diverse genus and fragmented microbial community in the Diarrhea group, while the Healthy group exhibited fewer but more dominant bacterial genera. For instance, the Healthy group showed higher relative abundances of beneficial genera such as *Bifidobacterium* (28.72%), *Faecalibacterium* (8.32%), *GAG-317*-143701 (9.38%), *Blautia* (1.8%), and *Akkermansia muciniphila* (1.85%). In contrast, those genera were diminished in the Diarrhea group, with respective abundances of 19.57%, 0.2%, 0.3%, 0.031%, 13.1%, 3.1%, and 0.8%, respectively. Conversely, the diarrhea group showed increased levels of other genera, for example, *Bacteroides* (6.4%), *Imtechenella* (5.59%), and *Collinsella* (5.23%), compared to the healthy group, which had 1.6% for *Bacteroides*, 0.11% for *Imtechenella*, and 3.89% for Collinsella. Importantly, other bacteria constitute a large and dominant proportion of the total microbiota in the Diarrhea group. This suggests that the summing of many minor or unclassified taxes has proliferated, leading to a less structured community. The remaining genera in the diarrhea group are present at much lower, more uniform levels, indicating a lack of dominant beneficial bacteria and a general collapse in community structure.

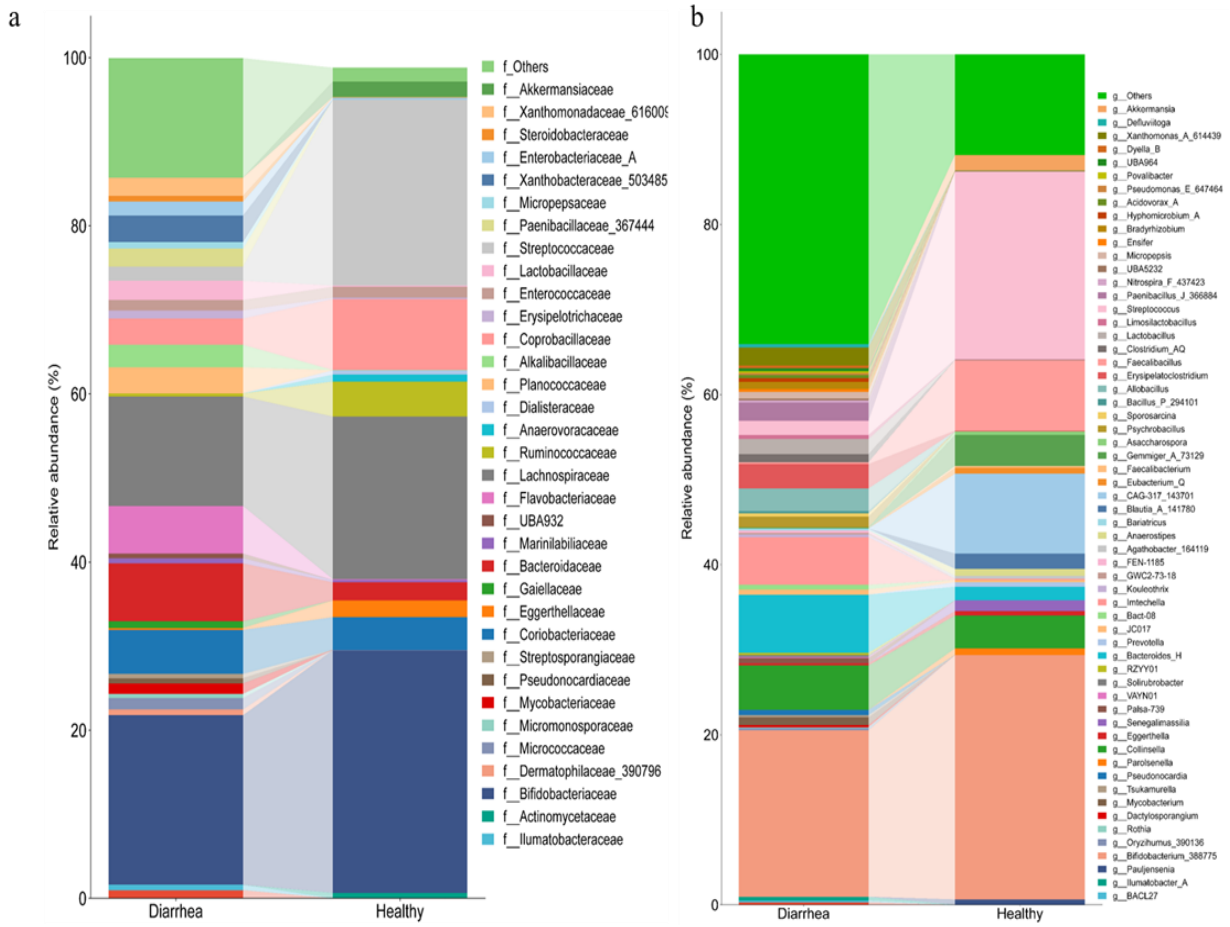

Figure. 2: Relative abundance of gut microbiota at the family (a) and genus (b) levels in Diarrhea and Healthy groups. Stacked bar plots show the mean relative abundance of individual taxa, with each color representing a distinct bacterial family or genus.

The Diversity and Hierarchical Clustering of the Bacterial Communities:

The diversity of taxa between healthy samples and those with diarrhea was analyzed using both α-diversity and β-diversity metrics. Regarding α-diversity metrics, although diarrhea samples exhibited non-consistent diversity than healthy samples for all α-diversity metrics, however, there were no significant statistically difference observed. For instance, Chao1, Simpson, and Index ACE (Figure 3a). For β-diversity, differences in bacterial communities between diarrhea and healthy individuals were evaluated using the Bray–Curtis index, the Jaccard index, and the Jensen–Shannon divergence (Figure 3b). All three β-diversity measures demonstrated a clear clustering of healthy samples, with a distinct separation from diarrhea samples. Further analysis of these taxa between healthy samples and those with diarrhea was performed using a hierarchical clustering dendrogram (Figure 3c). All healthy samples (H1–H4) form a tight cluster, indicating high similarity in their gut microbiota composition. Most diarrhea samples (D1, D2, D4, D5, D3, D6) cluster separately from the healthy group, suggesting that diarrhea is associated with a distinct microbial community structure.

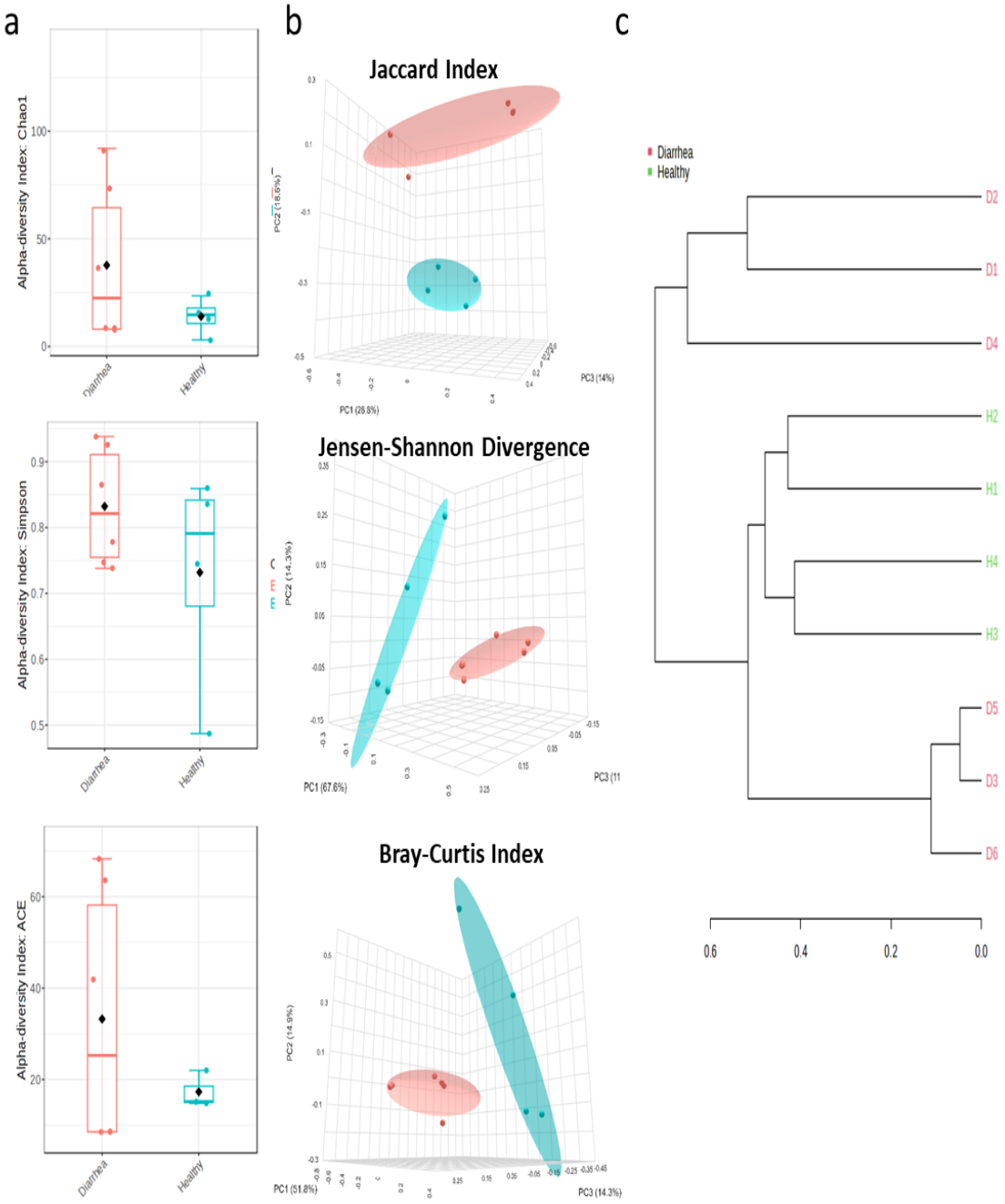

Figure. 3: The α- diversity (a), and β-diversity (b) metrics and hierarchical clustering dendrogram (c) comparing gut microbiota between Diarrhea and Healthy groups.

Box bar plots showing the difference and higher metrics indices in Diarrhea (red) compare with Healthy (blue) groups and these are (Chao1, Simpson and ACE) (a). 3D PCoA plots for visualized β-diversity analysis metrics (Jaccard, Jensen–Shannon divergence, and Bray–Curtis) showing clear separation between groups (b). Dendrogram comparing the gut microbiota profiles of individuals with diarrhea (labeled D1–D6) and healthy individuals (labeled H1–H4).

Heat Tree Analysis:

The Metacoder Tree was used for summarizing the significant difference of microbial taxa between the Diarrhea cases and Healthy groups. Within the *Verrucomicrobiota* phylum (p = 0.03), a marked reduction was observed at the *Verrucomicrobiae* family level (p=0.03), particularly in the *Akkermansia* genus. In contrast, the *Bacteroidota* phylum showed a remarkable increase (p= 0.01), driven mainly by the *Bacteroidia* class (p = 0.01), and the *Prevotella* genus (p=0.026).

Although no significant changes were detected at the phylum level for *Actinobacteriota*, *Proteobacteria* and *Patescibacteria*, several lower-level taxa within these phyla exhibited notable shift. Within *Actinobacteriota* phylum, there *Thermoleophilia* class (p=0.023) and its order *Gaiellales* (p=0.031) were significantly increased, as were the order *Mycobacteriales* (p=0.019) and the family *Mycobacteriaceae* (p =0.019). Moreover, within *Actinomycetales* order, the *Micrococcaceae* family also showed an upward trend (p=0.14), while the *Actinomycetaceae* family declined (p=0.17), driven by reductions in both genera, *Pauljensenia* (p=003) and *Peptidiphaga* (p=0.03). Within *Proteobacteria* phylum, there was a remarkable increase of *Micropepsis* genus (p=0.03), belonging to the *Micropepsaceae* family (p=0.031), and the *Micropepsales* order (p=0.031). In contrast, the *Patescibacteria* phylum, showed a reduction in the SDRW01 genus (p=0.03) from UBA10027 family (p=0.03).

In addition to these phyla also, although there is no significant difference were observed at the phylum level for both *Firmicutes* A and *Firmicutes* C, several lower-taxa within these *Firmicutes* phyla displayed substantial shifts. In Firmicutes_A, the *Clostridia*_258483 class showed remarkable reductions across multiple genera within *Lachnospiraceae* family from *Lachnospirales* order. These genera include: CAG-317_143701 (p=0.009), *Anaerostipes* (p=.006), *Blautia*_A_141780 (p=0.03), *Eubacterium*_Q (0.03), *Schaedlerella* (p=0.03) and *Agathobacter*_164119 (p=0.05). Additionally, decreases were observed from the *Peptostreptococcales* order, particularly *Anaerovoracaceae* family (p=0.03) and two genera from the *Peptostreptococcaceae*_256921 family, *Asaccharospora* (p=0.03) and CCUG-7971 (p=0.05). Within *Oscillospirales* order, reductions were evident in *Bittarella* (p=0.03) from *Ruminococcaceae* family, and *Faecalibacterium* (p=0.03) form *Ruminococcaceae* family, as well as *Clostridium*_P (p=0.03) from *Clostridiaceae*_222000 family. In *Firmicutes*_D, significant changes were also noted. An unclassified family within *Lactobacillales* order was reduced (p=0.03), alongside shifts in several genera across different families; *Gemella* (p=0.03) from *Gemellaceae* family (p=0.03), *Catenibacterium* (p=0.03) from *Coprobacillaceae* family, and *Bulleidia* (p=0.05) from *Erysipelotrichaceae* family.

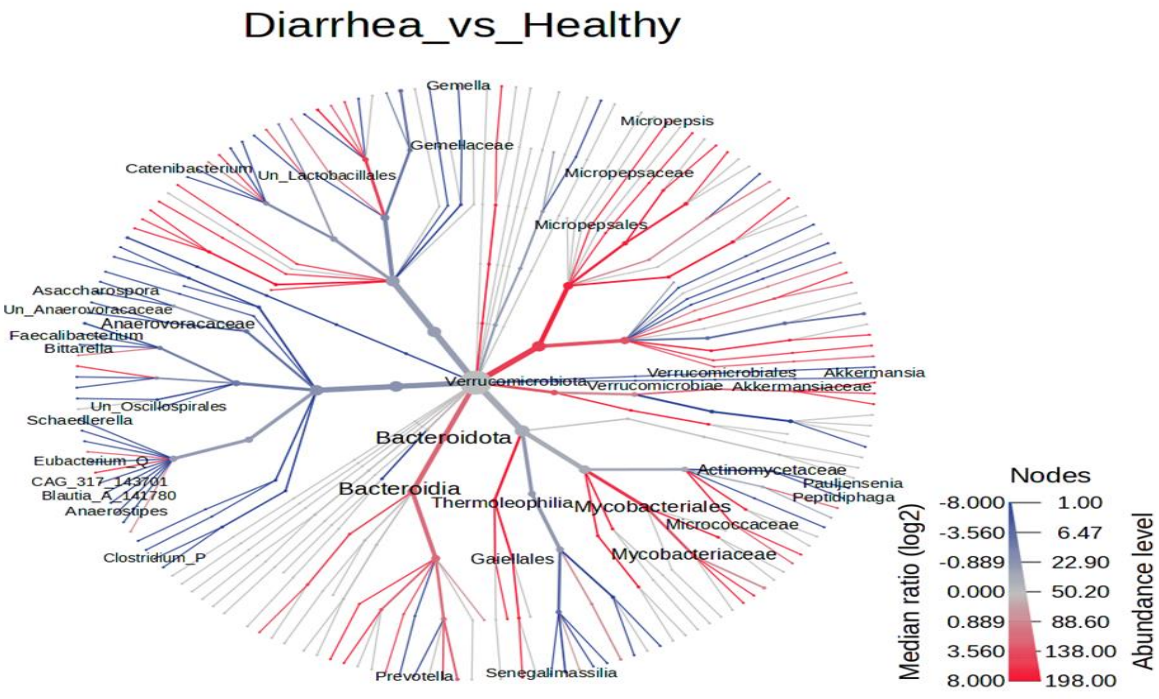

Figure 4: Heat tree analysis of bacterial taxa between Diarrhea and healthy individuals.

4. DISCUSSION

The gut microbiome is a dynamic ecosystem central to health, regulating metabolism, mucosal immunity, and pathogen resistance. The current study supports several well-established features of diarrheal dysbiosis. Consistent with previous reports, diarrheal samples were enriched in *Proteobacteria*, a phylum widely recognized as a marker of gut microbial imbalance (Ibrahim *et al*., 2020; Kang *et al*., 2019; Ku *et al*., 2025; The & Le, 2022). Family members of this phylum, including *Enterobacteriaceae*, expand under inflammatory conditions, exploiting increased oxygen and nitrate availability to worsen epithelial injury and disease severity (Wu e*t al*., 2022; Xi *et al*., 2021). Equally, there was a decrease in *Firmicutes* subdivision taxa, which are largely responsible for fiber degradation and short-chain fatty acid (SCFA) production. Recently, in modern taxonomic frameworks such as Genome Taxonomy Database (GTDB), the *Firmicutes* phylum was subdivided into five clades rather than being treated as a single monophyletic phylum according to nomenclature, and this is based on *Firmicutes* lineages into distinct phylogenetic clades (McDonald *et al*., 2024; Parks *et al*., 2026). The depletion of these taxa has been consistently associated with reduced butyrate supply, compromised intestinal barrier integrity, and altered host energy harvest (An *et al*., 2023; Li *et al*., 2021; Zhuang *et al*., 2018). At finer resolution, the reduction of *Ruminococcaceae* and *Lachnospiraceae* and the concurrent increase in *Enterobacteriaceae* and *Enterococcaceae* replicate global findings in diarrheal and critically ill populations (George *et al*., 2022; Li *et al*., 2021; Schlechte *et al*., 2023). *Streptococcaceae* abundances were observed in other pediatric populations with diarrhea and might represent a marker of microbial imbalance (Kieser *et al*., 2018). Usually, this group increases during diarrhea, but our data shows the opposite. The increase in this family might be due to a favorable environment characterized by alterations in oxygen and nutrient availability, which promote the growth of this group of facultative anaerobic bacteria (Kieser *et al*., 2018). However, some studies show variation in species abundance; for example, it has been noted that the ratios of some *Streptococcus* species, such as *S. salivarius*, declined during recovery (Jin *et al*., 2013). The same was reported in individual cases in which variation in *Streptococcaceae* during different stages was observed (The & Le, 2022). This current study also identified reduced *Akkermansiaceae*, particularly *Akkermansia muciniphila*, a taxon repeatedly linked to mucus-layer maintenance and epithelial protection (Hernández *et al*., 2019; Z. Wu *et al*., 2022). In addition, within the *Bacteroidota* phylum, functional decline may be masked: favorable, beneficial SCFA-producing species, such as *Prevotella* expand, could be diminished. Indeed, a significant elevation of *Bacteroidota/Firmicutes* and *Proteobacteria/Firmicutes* ratios in diarrhea samples, with wider variability compared to controls. These ratios have been proposed as integrative indicators of dysbiosis and may serve as candidate biomarkers for distinguishing between healthy and diarrheal states (An *et al*., 2023; Stojanov *et al*., 2020). This suggests that diarrhea not only shifts the microbiota away from the healthy state but also introduces more variability among affected individuals (Wu *et al*., 2022).

On the other hand, loss of dominant commensals may open ecological niches that allow opportunists to proliferate, inflating richness, increasing diversity, without indicating true stability (An *et al*., 2023; Stojanov *et al*., 2020). Inflammatory processes generate heterogeneous gut microenvironments that can sustain a broader taxonomic representation. Despite these divergences, β-diversity analyses revealed that healthy samples were tightly clustered together, reflecting similar gut microbiota composition, whereas diarrheal samples were more dispersed and formed distinct groups. In addition, hierarchical clustering of healthy samples forms a tight cluster, indicating high similarity in their gut microbiota composition, while all diarrhea samples cluster separately from the healthy group. These suggest that diarrhea is associated with a distinct microbial community structure. These findings are aligning with previous studies that diarrhea disrupts gut microbial composition, reduces similarity to healthy controls, and increases inter-individual variability (An *et al*., 2023; Stojanov *et al*., 2020; Wu *et al*., 2022; Zhuang *et al*., 2018). In addition, diarrheal children exhibited a loss of beneficial SCFA-producing taxa, including *Blautia*, *Faecalibacterium*, and *Bifidobacterium*. These genera are central to butyrate production, gut barrier support, and immunomodulation (Gallardo *et al*., 2020; Holmberg *et al*., 2024; Iancu *et al*., 2023; Sun *et al*., 2019). Their depletion was accompanied by the proliferation of minor or unclassified genera, resulting in a fragmented community structure. This pattern has also been observed in Ethiopian and Chilean children with diarrhea, where reductions in commensals coincided with opportunist expansion (Iancu *et al*., 2023; The & Le, 2022). The functional implications are considerable, as diminished SCFA production weakens colonization resistance, reduces anti-inflammatory signaling, and may prolong or worsen diarrheal episodes.

However, an increase in *Bacteroidota* in diarrheal samples was observed, whereas most previous studies reported decreases (An *et al*., 2023; Ku *et al*., 2025). Several explanations may account for this discrepancy. Dietary context is critical; Kurdish children consume fiber-rich, plant-based diets that support saccharolytic *Bacteroidota* even under diarrheal stress. Age-specific dynamics may also contribute, since children aged 4–6 years often exhibit high natural *Bacteroidota* dominance compared to infants or adults studied elsewhere (Stojanov *et al*., 2020). Deering *et al*., in reviews study mentioned that by the age 4, the gut microbiota composition become more complex and the composition may influenced by geographical area, culture, age and methodology (Deering *et al*., 2019). Furthermore, it may be due to technical factors such as small sample size, sequencing depth and normalization may exaggerate richness estimates. Similar anomalies have been described in animal models of diarrhea, where increased diversity reflected instability rather than resilience (Sun *et al*., 2019). In addition, differences in disease stage at the time of sampling may also explain variation, as communities may reorganize

differently in acute versus convalescent (An *et al*., 2023; Stojanov *et al.*, 2020). Diarrheal dysbiosis is not monolithic; rather, it reflects both universal hallmarks and local contingencies.

Overall, these data highlight several global indicators of microbial dysbiosis in patients with diarrheal disease. These variations may be explained by dietary habits, ecological compensation, age-related microbiome maturation, inflammatory ecology, and methodological limitations. Even though this study was limited by a small sample size and reliance on 16S rRNA amplicon sequencing targeting the V3–V4 regions only, it underscores the importance of regional investigations. In addition, recruitment from a single pediatric hospital in Duhok limits the generalizability of the finding.

CONCLUSION

This research provides evidence of a clear alteration in the gut microbiome in pediatric with diarrhea. This alteration is primarily characterized by an increase of opportunistic taxa, for instance Proteobacteria, and a decrease in some beneficial commensals such as *Blautia*, *Faecalibacterium Bifidobacterium*, and *Akkermansia muciniphila*. Likewise, significant shifts in the ratio between phyla, especially a shift increase of the *Bacteroidota/Firmicutes* and *Proteobacteria/Firmicutes* ratios. Additionally, β-diversity analyses confirmed distinct clustering and imbalance in the bacterial community composition with higher variability among samples. To sum up, the outcomes highlight the prominent roles of microbiome dysbiosis in childhood diarrhea. These emphasize those taxa to impair the metabolic function in the gastrointestinal tract such as SCFAs production alongside with causing leaky gut, and this is potentially contributing to the pathogenesis. Further studies for longer, larger and different methods of metagenomic studies are warranted to not only validate these findings but also to explore microbiota-targeted interventions. Additionally, the study highlights the importance of region-specific investigation into pediatric diarrheal microbiome.

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Ethical Approval:

This study was approved by the Research Ethics Committee of the Duhok Directorate General Health (No.13072021-7-7). Written informed consent was obtained from each child's parent prior to sample collection.

Author Contribution:

Conceptualization, A.Y.S. and K.S.I.; methodology, B.N.A. and A.Y.S.; software, K.S.I.; validation, A.Y.S. and K.S.I.; formal analysis, K.S.I.; investigation, BA.; resources, B.N.A. and A.Y.S.; data curation, O.E.E. and I.K.; writing original draft preparation, A.Y.S. and K.S.I. writing review and editing, A.A., Z.N.A. and K.S.I.; visualization, K.S.I.; supervision, A.Y.S. and K.S.I.; project administration, A.Y.S. All authors have read and agreed to the published version of the manuscript.

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