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Ironing Out Anemia: High Inflammation Modulates Microbial Composition and Indicates the Activation of Cellular Stress Responses in Infected Anemic Infants

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SUMMARY Iron Deficiency Anemia (IDA) is a significant global health challenge impacting cognitive, physical, and behavioral development, particularly in infants. Despite the current knowledge on anemia and its manifestations, the role of the gut microbiome and its relationship with systemic inflammation and anemia have yet to be explored. This study explores the relationship between anemia, systemic inflammation, and the gut microbiome in infants using a dataset from McClorry et al. Our analysis revealed that neither infection status nor inflammation levels significantly altered microbial diversity, however, differentially abundant genera were related to inflammation levels in infected anemic infants. Metabolic pathways analysis further identified the upregulation of pathways involved in energy production and immune response, emphasizing the systemic effects of inflammation in anemic infants. Our findings underscore the potential of microbial interventions in managing inflammation-related anemia, highlighting the need for further research into the microbiome's role in inflammatory processes.

INTRODUCTION

Iron is a significant micronutrient in the human body essential for growth and development (1). The element plays a critical role in cell function and proliferation, erythropoiesis, and oxygen transport (1). However, iron deficiency is a serious public health issue worldwide, particularly in developing countries where staple foods contain low iron and nutritional diversity is less accessible (2). Anemia can result from inadequate iron intake, which accounts for more than half of all anemia cases (3). Prolonged iron deficiency can result in iron deficiency anemia (IDA) (4), affecting around 16% of the global population and disproportionately impacting women and children under 5 years of age (5). IDA is characterized by inadequate iron storage or retention in the body and is often exacerbated by inflammation, leading to severe cognitive, physiological, and behavioral consequences (4).

Ferritin, a primary iron storage protein, is increasingly recognized as an acute-phase reactant and an indicator of inflammation in studies involving the gut microbiome. Elevated ferritin levels are often observed in response to systemic inflammation, serving as a protective mechanism to sequester iron from pathogens, thus limiting their growth (6, 7). Research has shown that changes in ferritin levels can reflect the inflammatory status influenced by gut microbiota dysbiosis or disorders (8), making it a valuable biomarker for assessing gut-related inflammatory conditions (7).

Various studies have demonstrated how gut dysbiosis — an imbalance in the microbial community — can contribute to anemia by impacting iron absorption and utilization (1). For instance, an overgrowth of pathogenic bacteria that compete for iron can reduce the quantity available for host absorption (9). Conversely, beneficial bacterial species such as

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Lactobacillus and *Bifidobacterium* have been associated with enhanced iron absorption and utilization (4), underscoring the potential of the gut microbiome in mitigating IDA (10). Anemia, particularly prevalent in infants, can be compounded by infections that alter gut microbial composition and function, leading to impaired iron absorption and utilization. Moreover, certain infections can induce inflammation, which further affects iron metabolism by increasing ferritin levels, a process that sequesters iron away from both the host and pathogens (11). As such, this complex interaction between infection, the gut microbiome, and anemia necessitates further exploration to provide greater insight into addressing the underlying causes.

Our study pivots towards understanding the complex interplay between anemia, systemic inflammation, and the gut microbiome. By measuring adjusted ferritin levels, we can discern the extent of inflammation, which is a common complicating factor in anemia. This approach allows for a more accurate assessment of the iron status and the inflammatory state of the patient, providing a clearer picture of the interplay between anemia, inflammation, and gut microbiome alterations. Specifically, our study uses adjusted ferritin levels as a marker of inflammation to explore how high levels of inflammation in anemic infants can modify the gut microbial composition and trigger cellular stress responses. By examining the differential abundance patterns in the microbiomes of infected anemic infants with varying levels of inflammation, we seek to identify key microbes that underpin these relationships. Additionally, our study will investigate the metabolic pathways activated in response to inflammation, offering new insights into the systemic effects of anemia and identifying potential microbial targets for therapeutic intervention.

We hypothesize that alterations in the gut microbiome composition, driven by inflammation, contribute to the pathogenesis of anemia in infected infants. Specifically, we expect to observe shifts in microbial abundance and metabolic activity associated with high inflammation status, potentially exacerbating anemia and impacting overall health outcomes.

METHODS AND MATERIALS

Dataset and metadata. The dataset utilized for this study was originally generated by McClorry et al. from the University of California, Davis (12). It includes cross-sectional stool and serum samples collected from a cohort of 95 infants (53 maleand 42 female), all aged 12 months and drawn from the broader Moronacocha area in Iquitos, Loreto, Peru. Stool samples underwent DNA extraction and 16S ribosomal RNA gene Illumina sequencing, focusing on the V4 region amplified using the F515-R806 primer pair. Fecal and serum metabolomes were quantified using 1H-nuclear magnetic resonance. Anemia was defined according to the World Health Organization (WHO) with a hemoglobin concentration <120 - 130 g/L (13). Infection status was further classified based on standard cut-offs of serum C-reactive protein (CRP) >5 mg/L and α1-acid glycoprotein (AGP) >1 g/L, categorizing infants into groups: incubation (elevated CRP only), early convalescence (elevated CRP and AGP), late convalescence (elevated AGP only), and reference (neither elevated). Incubation, early convalescence, and late convalescence were grouped to represent “infected” infants. Ferritin levels (adj_ferritin_status) were also reclassified, with normal levels classified as high inflammation and deficient levels classified as low inflammation.

Developer platform. GitHub was used as the main developer platform for this project to create and store code. The GitHub repository can be found here: https://github.com/Kshemaka1/Team-12-Jaydens-475-Crew

Data processing in QIIME2. The sequencing data was processed using the Quantitative Insights Into Microbial Ecology Version 2 (QIIME 2) platform (14) which is detailed in the supplemental QIIME2 Script (QIIME2Script). Single-end sequence reads were imported using the manifest format and demultiplexed to resolve individual sample data. The reads underwent a quality control process using the Divisive Amplicon Denoising Algorithm 2 (DADA2), which corrects errors in amplicon sequencing and identifies unique amplicon sequence variants (ASVs) (15). The sequences were truncated at 253 base pairs, a length determined to ensure a minimum median Phred score of 30 (Figure S1). Post-quality control,

the reads were denoised, and chimeric sequences were removed. The non-chimeric sequences were clustered into ASVs, resulting in a total of 193 samples and 1434 distinct features.

Taxonomic analysis in QIIME2. Taxonomic classification for the samples was generated using the SILVA 138-99 database (16). QIIME2 was utilized to eliminate any mitochondrial and chloroplast DNA sequences present in the samples. A phylogenetic tree was constructed using QIIME2 FastTree and MAFFT alignment methods (14).

Metadata filtering in R. OTUs with low significance (counts < 5 across samples) and samples with insufficient read depth (< 100 reads) were excluded. Data was subsetted to include only 12-month-old infants diagnosed with anemia. Filtered data to include only samples with infected infants. Metadata filtering is detailed in the supplementary R script (RScript 1).

R packages used. Analysis was done using R version 4.3.2 and the following R packages were installed and loaded: tidyverse (17), DESeq2 (18), dplyr (19), vegan (20), ggplot2 (21), phyloseq (22), ape (23), microbiome (24), and indicspecies (25).

Alpha and beta diversity analysis. To address differences in sequencing depth across samples, the dataset was rarefied to a depth of 10,000 sequences per sample. Alpha diversity was assessed using Shannon's diversity index. A Wilcoxon rank sum test was carried out to determine statistical significance between infected anemic infants with high and low inflammation. Shannon's diversity was also carried out on the infection status (incubation, early/late convalescence) of anemic infants, and significance was tested with a Kruskal-Wallis test. Alpha diversity analysis is detailed in the supplemental R script (RScript 1 & RScript 2). Beta diversity was assessed through weighted unifrac distance matrices between infants with high and low inflammation. Results from beta diversity analysis were visualized by generating a Principal Coordinates Analysis (PCoA) plot. Permutation multivariate analysis of variance (PERMANOVA) was performed to assess statistical significance. Visualization of data was performed using ggplot2 (21). Beta diversity analysis is detailed in the supplementary R script (RScript 3).

Differential Abundance Analysis. Differential abundance analysis was conducted on infected infants with high and low inflammation levels to identify changes in the abundance of shared genera. In this analysis, infected infants with low levels of inflammation were used as the reference group. This analysis was carried out using the DESeq2 package (18). All p-values were adjusted using the Benjamini–Hochberg procedure via the DESeq2 package (18). Genera exhibiting significant abundance changes from the volcano plot were further visualized using a bar chart, highlighting both upregulated and downregulated genera. The visualization of data was performed using ggplot2 (21). Differential abundance analysis is detailed in the supplementary R script (RScript 4).

Core Microbiome Analysis. Core microbiome analysis was conducted using the microbiome R package (24). Relative abundance for each inflammation group (high vs. low) was derived from the phyloseq object. The core microbiome analysis was performed using the microbiome package, with detection and prevalence thresholds set to 0 and 0.7, respectively (24). A Venn diagram was then created using ggVennDiagram to illustrate the overlap of core taxa between the two groups, quantifying both the number of shared taxa and their corresponding percentages (21). Core microbiome analysis is detailed in the supplementary R script (RScript 5).

Functional Analysis using PICRUSt2. To conduct functional analysis, the DESeq2 package in R was utilized (18). Data was processed using the DESeq2 algorithm, specifically focusing on differential abundance and expression across high and low inflammation levels. Statistical evaluation of the microbiome data identified pathways and enzymes where p-values < 0.05 were considered statistically significant. For enzymes, only those with a log2 fold change > 4 were included. Both significant pathways and enzymes were annotated using the MetaCyc

database (26). The visualization of data was performed using ggplot2. Functional analysis is detailed in supplementary R script (RScript 6 & RScript 7).

RESULTS

Inflammation did not influence the microbial diversity of the infant gut microbiome. To determine whether there was a difference between stages of infection (early convalescence, late convalescence, and incubation) and levels of inflammation (high or low) in anemic infants, we carried out an alpha and beta diversity analysis using the Shannon Diversity Index and Weighted Unifrac analysis respectively. The Shannon Diversity Index revealed that there was no difference in diversity between early convalescence, late convalescence, and incubation infection stages (Figure 1A), nor was there a difference between high and low inflammation levels in anemic infants (Figure 1B). Due to the lack of alpha diversity difference between each infection status, all infection stages were grouped into one "Infected" category for the beta diversity analysis. Weighted Unifrac analysis and a resulting PCoA plot demonstrated that there was no difference in diversity between low and high inflammation infected anemic infants (Figure 2). Our findings indicate that the diversity of fecal microbes remains consistent throughout active infection and is not notably influenced solely by the stages of infection.

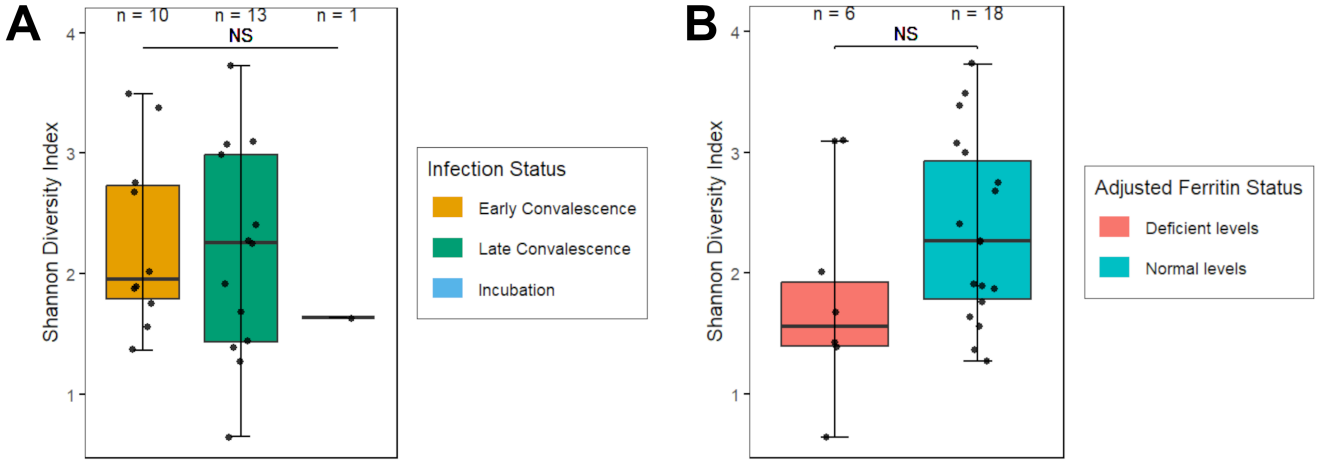

FIG. 1 No significant difference in alpha diversity across different stages of infection and adjusted ferritin status in 12-month-old infected anemic infants. **A.** Fecal microbial alpha diversity using Shannon's Index among patients at different infection stages: early convalescence (orange), late convalescence (green), and incubation (blue). No significant difference was observed (NS) across stages (Kruskal-Wallis test). **B.** Fecal microbial alpha diversity was measured using Shannon's index across patients with different inflammation levels: low (red) and high (blue). No statistically significant differences observed among groups (Wilcoxon rank test).

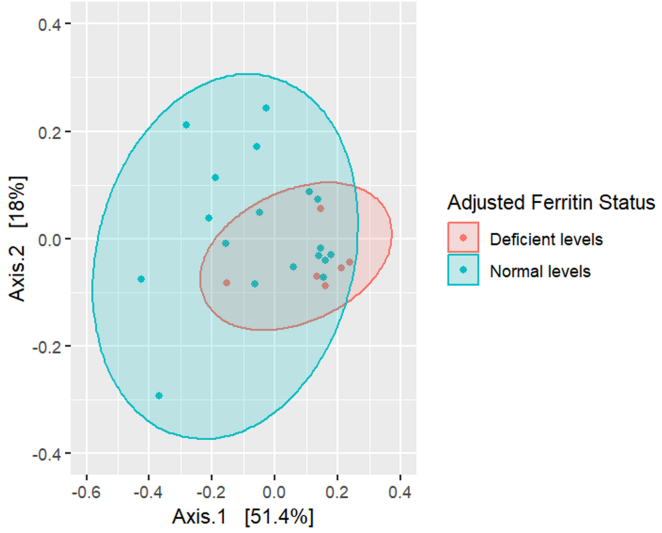

FIG. 2 No significant difference in beta diversity between 12-month-old infected anemic infants with high and low inflammation levels. Principal Coordinates Analysis (PCoA) plot based on Weighted UniFrac distances showing the beta diversity between 12-month-old infants with high and low inflammation levels. Samples are categorized into two groups: low inflammation (red) and high inflammation (blue). Each point represents the microbial community of an individual infant. Ellipses represent the 95% confidence intervals. No statistically significant differences between the two groups as determined by PERMANOVA.

Bifidobacterium exhibited decreased abundance in infected anemic infants with high inflammation. Differential abundance analysis was performed on the subset of infected anemic infants (n = 25) using DESeq (18) and represented in a volcano plot (Figure 3A) and bar chart (Figure 3B). Among the various genera, 19 demonstrated significant upregulation in the gut microbiota of infected anemic infants with high inflammation levels compared to those with low inflammation levels, while only Bifidobacterium demonstrated significant downregulation in the gut microbiota of infected anemic infants with high inflammation levels. Collectively, this suggests a drastic shift in microbial composition within the gut environment as a result of inflammatory levels.

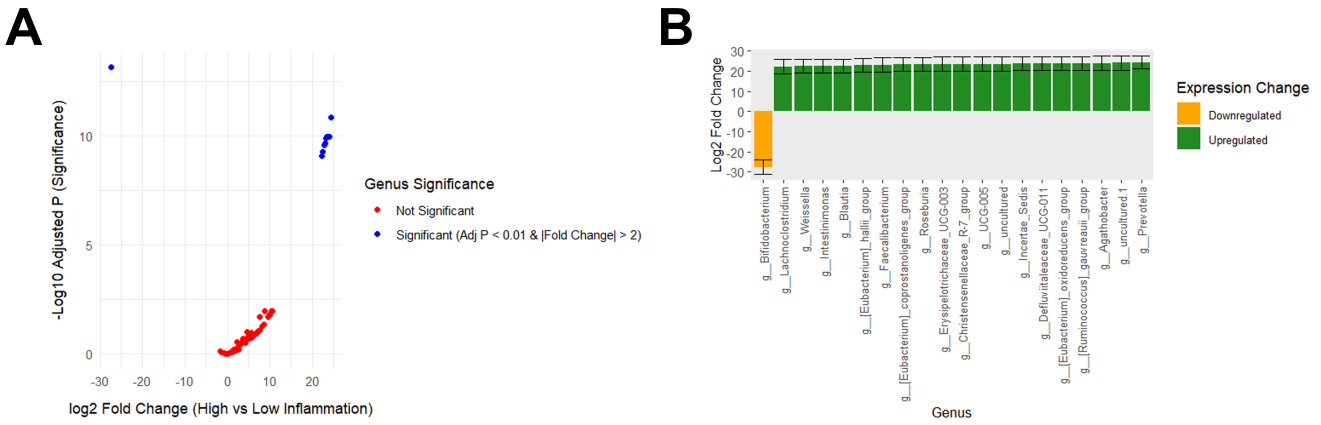

FIG. 3 Distinct Microbial Profiles in Low vs. High Inflammatory States of the Gut A. Volcano plot highlighting significant and non-significant differential genera expression with fold change in infected anemic patients with high inflammation to low inflammation. Significantly upregulated or downregulated genera are shown in blue (Adj P < 0.01 and |fold change| > 2) and not significantly upregulated and downregulated genera are shown in red. **B.** Genus-Level Differential Expression Profile. Bar chart indicates fold changes in significant genus-level expression in infected anemic patients with high inflammation relative to low inflammation. Upregulated genera are shown in green, and the sole significantly downregulated genus is displayed in orange.

Alterations in gut microbiota composition correspond to inflammation status in infected anemic infants. To assess whether there was a difference in the abundance of bacterial genera between low and high inflammation states in infected anemic infants, the relative abundance of bacterial taxa was explored (Figure 4). Across our samples, we observed a general increase of species within the *Ruminococcus*, *Bacteroides*, *Enterococcus*, *Escherichia-Shigella*, and *Lactobacillus* genera in patients characterized with high inflammation status relative to low inflammation status (Figure 3). Additionally, *Bifidobacterium* appeared to be less abundant in patients with a high inflammation status, which corresponds to the findings of our differential abundance analysis (Figure 4). This suggests a potential dysbiosis associated with inflammation in infected anemic infants.

Distinct gut microbial profiles in low and high inflammation states of infected anemic infants. A core microbiome analysis was conducted to determine which abundant taxa of the gut were unique or common to low and high inflammation conditions (Figure 4B). Present in at least 70% of the samples, three amplicon sequence variants (ASVs) were found to be unique for low inflammation status whereas two ASVs were found to be unique for high inflammation status. Additionally, four shared ASVs suggest a degree of similarity in the microbial composition of both conditions. Further examination of these taxa (Table 1), revealed *Lactobacillus mucosae* and an unclassified species within the *Enterococcus* genus, are solely associated with the low inflammation condition. Notably, the presence of *Bifidobacterium bifidum* also characterizes this less inflamed state. In contrast, an uncharacterized species within the *Faecalibacterium* and *Blautia* genera marks the high inflammation profile (Table 1). Among the taxa shared between patients with high and low inflammation, Bacteroides *fragilis*, *Ruminococcus gnavus* and a species within the *Escherichia-Shigella* genus was identified. Interestingly, an undefined species within the *Bifidobacterium* genus was also identified among the taxa common to both inflammation conditions (Table 1). Overall, the distinct presence of specific bacterial taxa in low and high

inflammation profiles suggests their potential role in the modulation of gut inflammation within infected anemic patients.

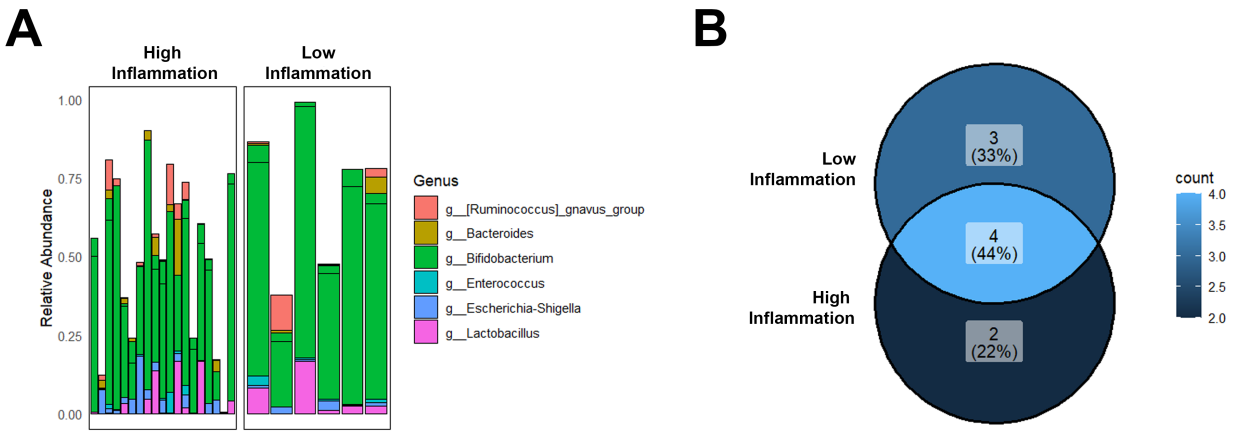

FIG. 4 Genera Abundance Variation and Exclusive Core ASVs in Anemic Patients with Low Inflammation. A. The relative abundances of key bacterial genera in infected anemic patients with high and low inflammation levels. **B.** Venn diagram highlights the exclusivity of two core ASVs to the high inflammation category, four that are shared amongst high and low inflammation groups, and three in the low inflammation category. Analyses were done using a 0% detection threshold and 70% prevalence.

TABLE. 1 Distribution of core microbiome in infected anemic infants classified by inflammation status. "Unique Low Inflammation" and "Unique High Inflammation" groups list taxa exclusive to infants with low and high inflammation markers, respectively. "Shared" indicates taxa found across both inflammation categories. Genera and species were identified using a 0% detection threshold and a 70% prevalence criterion, as depicted in the Venn diagram of Figure 2B. Instances where the dataset did not contain species-level classification within the dataset are denoted by "NA".

Genus	Species	Group
Lactobacilus	*Lactobacilus mucosae*	Unique Low Inflammation
Enterococcus	*NA*	Unique Low Inflammation
Bifidobacterium	*Bifidobacterium bifidum*	Unique Low Inflammation
Bacteroides	*Bacteroides fragilis*	Shared
Escherichia-Shigella	*NA*	Shared
Bifidobacterium	*NA*	Shared
Ruminococcus	*Ruminococcus gnavus*	Shared
Faecalibacterium	*NA*	Unique High Inflammation
Blautia	*NA*	Unique High Inflmmation

High inflammation triggered the upregulation of metabolic pathways/enzymes signaling stress and immune activation. Functional analysis was performed using PICRUSt2 (27) to compare metabolic pathways and enzymatic expression profiles for infected anemic infants with high and low inflammation (Figure 5). From this, numerous pathways and enzymes involved in energy production and immune activation were significantly upregulated in infected anemic infants with high inflammation relative to infected anemic infants with low inflammation, indicating substantial metabolic shifts. Some of the top enriched pathways in the high inflammation group included degradation pathways for energy such as L-histidine degradation II, glycogen degradation II, and nicotinate degradation I. Additionally, notable upregulated enzymes included proton-exporting ATPase, suggesting increased energy metabolism and potential immune activation in response to higher inflammation levels.

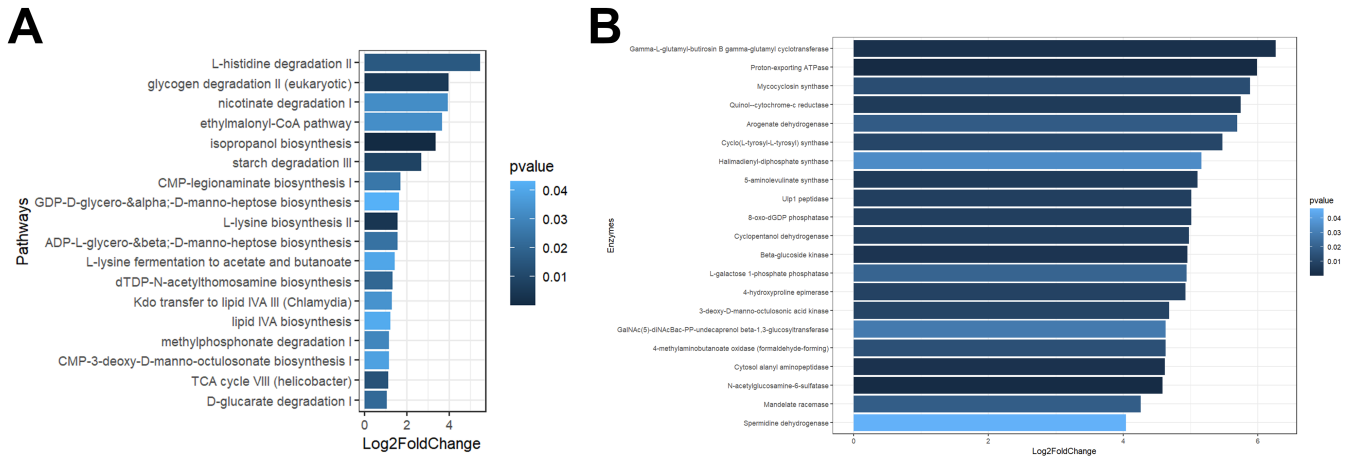

FIG. 5 Metabolic Pathways and Enzymes Significantly Upregulated in Infected Anemic Infants with High vs. Low Inflammation Levels. A. Ranked log2 fold changes in metabolic pathways for infants with high versus low inflammation levels with color depth indicating p-value significance. **B.** Significant log2 fold changes in enzyme expression, with darker hues indicating higher significance ($p < 0.05$), displayed for enzymes meeting the threshold (log2 fold change ≥ 4).

DISCUSSION

Infection stage and inflammation levels in anemic infants did not alter gut microbial diversity. Initial investigations aimed to use alpha diversity metrics to assess if microbial diversity across stages of infection and inflammation levels changed in anemic infants. However, no significant difference was seen between early convalescence, late convalescence, and incubation stages, nor when comparing high and low inflammation status. This is inconsistent with previous findings, which found an association between inflammation and alterations in both alpha and beta microbial diversity (28). It is plausible to attribute this discrepancy to the potential presence of gut microbiome functional redundancy whereby various microbes can fulfill similar roles to maintain stability against pathophysiological changes (29). Additionally, confounding variables such as the type of infection experienced by anemic infants is not clearly identified in the metadata, constraining possible deductions to the observed non-significance. Furthermore, the sample size of anemic infants in the incubation infection stage was small (n = 2), hindering the generalizability and representativeness of the group (12). As such, a larger sample size is necessary to validate our findings. Given this, downstream analyses grouped samples from all infection stages into one category, focusing on infants with infected anemic status.

Differentially abundant genera are related to inflammation levels in infected anemic infants. In our differential abundance analysis, *Bifidobacterium* was the only genus significantly downregulated which is distinct from the other genera that were significantly upregulated in infants with high inflammation (Figure 3B). *Bifidobacterium* is known for its anti-inflammatory properties, therefore this pattern may reflect a microbial shift towards a proinflammatory state within the gut environment of infected infants (30). The decline of *Bifidobacterium* in infected infants may exacerbate inflammatory processes which could potentially contribute to a more hostile gut environment (31).

The core microbiome analysis (Figure 4B) and core microbiome genus table (Table 1) indicated 3 unique genera found for low inflammation: *Lactobacillus, Enterococcus,* and *Bifidobacterium*, and 2 unique genera for high inflammation: *Faecalibacterium* and *Blautia*. *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* are all known for their probiotic and anti-inflammatory properties (32, 33, 34).

On the other hand, *Faecalibacterium* and *Blautia*, which are seen in the microbiota of high inflammation patients are known for providing anti-inflammatory properties and production of butyrate (35, 36). Butyrate is a short chain fatty acid that inhibits pro-inflammatory immune cells and activates anti-inflammatory immune cells, suggesting that

high inflammatory conditions promote the growth of these bacterial species to counteract inflammation (37).

Inflammation is linked to the upregulation of certain metabolic pathways and enzymatic expressions. A notable pattern of upregulation in both metabolic pathways and enzymatic expressions suggests a system adapting to inflammation's energetic and defensive demands. Increased metabolic activity was observed, with pathways related to the degradation of carbohydrates and amino acids experiencing a marked increase in expression (Figure 5). Upregulated glycogen and starch degradation pathways play an important role in rapidly mobilizing glucose stores—a critical fuel for the energy-intensive processes engaged by immune cells (38). Similarly, the increased expression of pathways involving L-histidine and L-lysine fermentation may indicate an elevated turnover of amino acids, likely providing for the increased demand for protein synthesis and additional substrates for gluconeogenesis during times of stress (39). Furthermore, the upregulation of the ATPase enzyme is indicative of an attempt to meet the high ATP demand characteristic of inflammatory states (40). ATPases, by hydrolyzing ATP, provide energy driving several cellular processes, from signal transduction to macromolecule synthesis. Their upregulation suggests an effort to boost ATP synthesis to potentially fuel the activities of the immune response, including the generation of immune mediators, cell proliferation, and migration (41).

Alongside these metabolic adaptations, the upregulation of protective mechanisms suggests a response to inflammation-induced stress. This is observed by the increased expression of the enzyme Gamma-L-glutamyl-butirosin B gamma-glutamyl cyclotransferase (Figure 5) which is involved in the biosynthesis of butirosin B, an aminoglycoside antibiotic that combats a wide range of bacterial infections (42). The heightened activity of this cyclotransferase in bacteria can be indicative of a response toward countering inflammatory processes and infection (43).

Limitations Our study presented several methodological and analytical limitations. First, the use of a small sample size limits the statistical power of the analyses and restricts the generalizability of our findings (44). Additionally, the dependency on single time point collection of stool samples fails to capture the day-to-day variability in the gut microbiome, which is especially variable in infants due to rapid changes in diet and immune development (12). This limitation may result in a perspective that is not reflective of the dynamics of the gut microbiome in relation to anemia and inflammation. Furthermore, ferritin level was used as the sole indicator of inflammation, however inflammation can be influenced by other factors such as presence of pathogens, illness, etc. that were not noted in the study (45). This approach may obscure other inflammatory pathways influential in pediatric anemia and gut microbiota interactions. Additionally, the study cannot provide information on species specificity, as samples were only sequenced to the genus level, limiting the precision of each analysis and potentially obscuring significant species-specific relationships to host health outcomes. Lastly, the functional analysis using PICRUSt2 remains a predictive approach and cannot be fully substituted for direct measurements of microbial metabolites or expression (46). Thus, conclusions about metabolic pathways and enzymatic activity are based on association rather than direct evidence of activity, which may not accurately reflect the true biological processes that occur within the gut microbiome.

Conclusions The objective of our study was to assess the impact of inflammation in anemic infants through the characterization of the gut microbiome. Although no significant differences in alpha and beta microbial diversity were observed between high and low inflammation states, differential abundance analysis identified a significant downregulation of the *Bifidobacterium* genus under high inflammation conditions. Core microbiome analysis highlighted the distinct genera in low inflammation levels (*Lactobacillus, Enterococcus*, and *Bifidobacterium*) and high inflammation levels (*Faecalibacterium* and *Blautia*). Furthermore, such microbial shifts were accompanied by the upregulation of stress and immune response-related pathways. Despite the non-significant finding of infection stages and inflammatory levels of anemic infants on gut microbial activity, the study demonstrates that inflammation in anemic infants likely influences the growth of specific bacterial genera and upregulates certain metabolic pathways and enzymatic expressions.

Future Directions Following this paper, studies regarding interventions, cross-population comparisons, and predictive modeling can be undertaken to gain a deeper understanding and application of the data surrounding infant anemia. Firstly, given the significant role that specific gut microbes, such as *Bifidobacterium*, may play in inflammatory processes and anemia in infants, intervention studies using probiotics or prebiotics could be a promising direction for future research. Targeting these interventions on the key microbial genera identified in this study could potentially modulate the gut microbiome in ways that lower inflammation and improve anemia outcomes. For example, preliminary studies in model organisms could be conducted to assess the efficacy and safety of interventions aimed at administering probiotics that boost beneficial bacteria or prebiotics that selectively promote their growth. These initial investigations could provide insights into the potential mechanisms and effects of such treatments before advancing to clinical trials in human subjects. Subsequently, clinical trials could be designed to evaluate the therapeutic potential of these interventions, closely monitoring microbial changes as well as changes in host metabolic and inflammatory indicators.

Our findings underline the importance of the gut microbiome in health outcomes related to inflammation and anemia. However, it is unclear how dietary, environmental, and genetic factors affect these relationships. Thus, future research should aim to investigate how the gut microbiota reacts to anemia and inflammation in diverse populations, including various age groups, ethnicities, and geographical regions. These explorations could help identify whether the trends seen in our cohort are general or exclusive to certain demographics. This could result in more personalized approaches to treating anemia and related conditions, potentially continuing targeted dietary or microbial therapies tailored to specific populations or regions.

Anemia and its complications pose significant health challenges, which are often made worse by inflammation. In relation to our current findings, there is a need to develop robust predictive models that integrate a broader array of metabolic indicators along with microbial composition data. These models could significantly improve early diagnosis and risk assessment, allowing for preventative or mitigative interventions. By incorporating advanced statistical and machine learning techniques, researchers could analyze complex datasets to predict the onset and severity of anemia. Furthermore, understanding how gut microbes interact with various host metabolites could discover new pathways and intervention, offering a systems-level perspective on the treatment of pediatric anemia. In clinical settings, these predictive tools would be invaluable in enhancing the ability to tailor interventions based on individual microbial and metabolic profiles.

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CONTRIBUTIONS

Ashnoor Arora (A.A), Ziniu Chen (Z.C), Kshemaka Gunawarden (K.G), Jayden Liu (J.L) and Jenny Zhang (J.Z) all contributed to script and data analysis on R and Rstudio. A.A, K.G, and J.L performed the data processing in QIIME2. All co-authors contributed equally to the writing, editing, and revision of the manuscript.

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