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Nutrient supplementation does not exacerbate pathogenic inflammation in infants with iron deficiency anemia

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SUMMARY Iron deficiency anemia (IDA) is a prevalent nutritional disorder with long-term negative cognitive, physiological, and behavioral effects, particularly in low-income and middle-income countries. Iron supplementation is the holistic strategy to treat IDA by replenishing iron stores and normalizing physiological hemoglobin concentrations. However, there is limited consensus towards whether iron supplementation can have therapeutic effects towards the clinical management of iron deficiency anemia. In this study, we compared the gut microbiome diversity between IDA infants receiving or not receiving iron supplementation, using 16S rRNA gene sequencing and bioinformatics analyses. We demonstrated that nutrient supplementation and infection status do not significantly affect microbial diversity. However, nutrient supplementation of infected anemic infants had beneficial gut microbiome changes and reduced pathogenic species. In sum, our study provides valuable insights into the effects of iron supplementation on the gut microbiome in infants with iron deficiency anemia, which could have significant implications in identifying interventions to improve the overall microbiome health of IDA infants.

INTRODUCTION

Iron deficiency anemia (IDA) and its association to the gut microbiota. IDA is a condition where there is either inadequate iron storage in the body or the retention of iron in the plasma is hindered due to inflammation (1). IDA is a major contributor to the global burden of disease, with a disproportionate impact on children, premenopausal women, and people living in low- and middle-income countries (LMICs) (1-2). It is a significant public health problem, affecting millions of infants, especially in low-income countries (1-2). Clinical presentations of iron deficiency include cognitive impairment, growth retardation, reduced concentration, and increased susceptibility to infections, leading to long-term cognitive, physiological, and behavioral problems (1-3).

The gut microbiome plays a crucial role in iron metabolism and absorption. Certain bacterial species in the gut microbiome have the ability to promote or hinder iron absorption in the gut (3-5). Previous studies have shown that gut dysbiosis, or an alteration in the composition of the gut microbiome, can contribute to iron deficiency anemia by affecting iron absorption and utilization. For example, dysbiosis in the gut microbiome can result in an overgrowth of harmful bacteria that compete with the host for iron, leading to a decrease in the amount of iron available for absorption. Conversely, some studies have suggested that certain beneficial bacterial species in the gut microbiome may enhance iron absorption and help prevent iron deficiency anemia (1-8). For instance, the presence of *Lactobacillus* and *Bifidobacterium* species in the gut has been associated with increased iron absorption and utilization (5-8). Since IDA is a prevalent deficiency worldwide and given that iron plays a critical role in shaping the gut microbiota, especially during early stages of infant development, studying alterations in systemic metabolism and fecal microbial diversity associated with iron deficiency anemia is of great interest (1-4).

Iron supplementation for infants diagnosed with IDA. Iron supplementation is the primary strategy for the prevention and treatment of IDA in infants. However, the impact of iron supplementation on the gut microbiome is not well understood. Iron supplementation can affect the gut microbiome in various ways, including the promotion of pathogenic species, reduction of beneficial species, increase in methane production, and formation of reactive oxygen species (3-6). Iron interventions in low-income settings, particularly in children, are complicated by the risk of infection. In areas where pathogenic bacteria are highly prevalent,

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iron may alter the composition of the intestinal microbiome, leading to an increase in pathogenic flora, consequently leading to infectious diarrhea.

In previous studies, there is evidence suggesting that IDA can alter the microbial structure and function in both sexes, but more so in males (2-4). For instance, in a recent study led by McClorry *et al.*, the authors studied how anemia and IDA in infancy affect metabolism and gut microbiome in a sex-specific manner. In their study, they found that anemia and IDA altered the microbial structure and function in both sexes, but more so in males (2). However, some gaps and limitations that the authors did not account for were potential confounders such as maternal nutrition, infection, or inflammation that may affect metabolism and gut dysbiosis. Moreover, the study did not focus on the effect of iron supplementation on gut microbiota health. This is especially crucial given that iron supplementation can affect the gut microbiota through several ways, including: promotion of pathogenic species, reduction of beneficial species, increase in methane production, and formation of reactive oxygen species (2-6).

Given the varying impact of iron supplementation on the gut microbiota of infants, the effects of iron supplementation on microbiome diversity in infants with IDA that are at different stages of infection have not yet been fully explored. To study this, we utilized a publicly available dataset published by McClorry et al., where the fecal microbiome of 95 infants at 12 months of age was analyzed based on microbial diversity among infants stratified into three different groups based on levels of C-reactive protein (CRP) and α1-acid glycoprotein (AGP) to classify infection status in the gut microbiome (early convalescence, late convalescence, and no infection). Indicator species analyses was also performed between different anemic subset groups to explore significant relationships between different sample groups and indicator species in the context of anemia and different infection conditions (2,4-6). Our study aims to fill these knowledge gaps of the interactions between infection status and supplementation in IDA patients by analyzing supplemented versus non-supplemented infants, as well as stratified IDA patients by infection status. Understanding how iron supplementation affects the gut microbial composition and function of infants is crucial, given that it is a common treatment for IDA.

The results of this study will provide insights into the effect of iron supplementation on the gut microbiome diversity of infants with IDA and help identify potential risks and benefits of iron supplementation. This information can inform the development of interventions to prevent and treat IDA while also promoting gut microbiome health in infants, particularly in low-income countries where IDA is prevalent.

METHODS AND MATERIALS

Dataset and metadata. Data for this study was obtained from McClorry *et al.* and compiled and formatted by the MICB475 teaching team (2). McClorry *et al.* gathered cross-sectional stool and serum samples collected from 95 infants (53 boys and 42 girls) at 12 months of age. The fecal microbiome was assessed by using 16S ribosomal RNA gene sequencing, and the fecal and serum metabolomes were quantified using 1H-nuclear magnetic resonance.
Anemia was defined as having a hemoglobin concentration <110 g/L as stated by the World Health Organization (WHO). Standard cut-offs of C-reactive protein (CRP) >5 mg/L and α1-acid glycoprotein (AGP) >1 g/L were used to classify infection status of each subject as either incubation (elevated CRP only), early convalescence (both elevated CRP and AGP), late convalescence (elevated AGP only), or reference (neither CRP nor AGP elevated). This information is summarized in the metadata and was used for analyses in our study. We grouped incubation, early convalescence, and late convalescence together to represent infected infants, and reference remained to represent uninfected, control infants. 12-month-old anemic infants were also grouped based on whether they take supplements, which included FeSO4 or micronutrient powders (MNP), or no supplementation.

Preliminary data processing in QIIME 2. We used QIIME 2 and DADA2 to import the reads and perform quality control (7-8). We demultiplexed, denoised, and clustered the reads using a truncation length of 253. This resulted in a total of 193 samples and 1434 features. Then, we conducted taxonomic analysis with a pretrained "silva-138-99-515-806-nb-

classifier.qza" classifier before removing mitochondrial and chloroplast sequences with QIIME 2. After rarefaction, a sampling depth of 12547 was determined and this was used to generate the alpha and beta diversity metrics. We decided to retain as many samples as possible, keeping 186 samples and 49% of the ASVs. Relevant .qza files were then converted and exported with commands "qiime tools export" and "biom convert" before further analysis using R. Further dataset filtering and subsetting would be performed in R (9).

R packages used. R version 4.2.1 was used for our analysis. We used ape, biomformat, DESeq2, dplyr, ggplot2, ggsignif, phyloseq, readxl, tidyverse, vegan, and indicspecies during our study to conduct analysis on QIIME 2 exported data (10-20).

Alpha diversity analysis of anemic infants. Microbial community richness within fecal samples from anemic infants were assessed by looking at alpha diversity. Shannon Diversity analysis was performed with QIIME2 and the resulting diversity metrics were exported to R for filtering and statistical analysis.

Analysis of fecal microbial diversity based on supplementation status. 12-month-old anemic infants were filtered based on their age and anemic status, then grouped based on whether they take supplements or not. Shannon's diversity analysis was performed between 12-month-old anemic patients who were taking iron supplements (n = 11) compared to those who were not taking supplements (n = 48). Wilcoxon rank sum exact test was performed to assess for significant differences. Visual representation of the data was performed using ggplot2 (10,18).

Analysis of fecal microbial diversity across varying infection statuses. Shannon diversity index metrics were merged with the original dataset for downstream analysis. The data was first filtered for anemic patients and then sorted based on infection status (reference, incubation, early/late convalescence) and then an ANOVA and Tukey range test was performed to assess for significant differences between the groups. Visual representation of the data was performed using ggplot2 (10).

Analysis of fecal microbial diversity across infected infants with or without iron supplementation. Shannon diversity metrics were merged with the original dataset for downstream analysis. The data was filtered for infected anemic infants (incubation, early/late convalescence). The data was then separated into those who took iron supplementation (MNP, FeSO4) and those that did not. Shapiro wilk test was used to assess for normality of the two groups and then a paired t-test was used to test for any significant differences in microbial diversity. Visual representation of the data was performed using ggplot2.

Indicator species analysis of anemic subset. For core microbiome analysis and indicator species analysis, the data was subsetted to include only anemic infants. The filtered data was then subsetted based on infection status ("Incubation", "Early convalescence", and "Late convalescence", or "Reference"). Between infected infants and uninfected infants, samples on supplements ("MNP" or "FeSO4") and not on supplements ("None") were compared. Infants with the infection status "Reference" are the uninfected control group. Using the "indicspecies" library, we compared the indicator species for anemic, uninfected infants on supplements; anemic, uninfected infants off supplements; anemic, infected infants on supplements; and anemic, infected infants off supplements.

RESULTS

Iron supplementation does not affect fecal microbial diversity in 12 months old anemic patients. Shannon Diversity analysis was employed in this study to explore the potential impact of iron supplementation on fecal microbial diversity in 12-month-old anemic patients. The results revealed no significant difference in microbial diversity between anemic patients receiving iron supplements and those who did not (Figure 1). This suggests that iron supplementation alone does not affect the microbial diversity within the gut microbiome of anemic patients. These findings indicate that other factors, such as diet or environmental

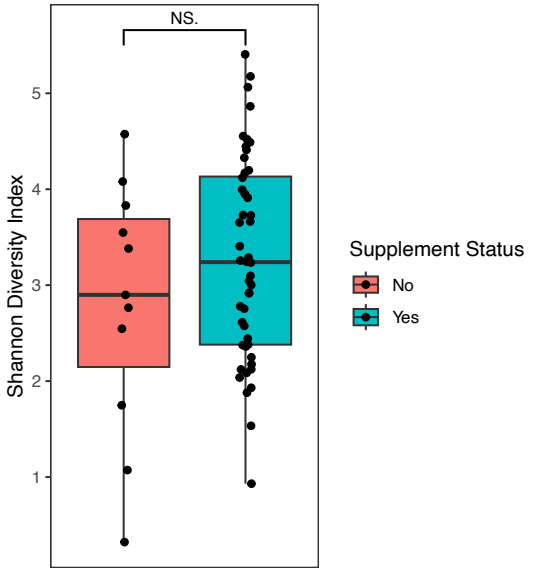

FIG. 1 No significant difference in effect of iron supplementation on fecal microbial diversity across 12-month-old anemic patients. 12-month-old anemic patients were grouped based on whether they take supplements in red (n=11), or do not take supplements in blue (n=48). Shannon's diversity analysis was used to assess fecal alpha diversity between two groups. Wilcoxon rank sum exact test shows no statistical significance between two groups (p=0.38).

exposure, may play a more significant role in shaping the microbial composition in anemic patients (1-3). Despite the lack of changes in microbial diversity, it is worth noting that iron supplementation has been shown to improve the growth and proliferation of certain bacterial taxa known to be beneficial for gut health, which may have additional positive effects beyond changes in diversity (1-5).

Infection status alone does not result in varying fecal microbial diversity within anemic patients. Shannon's Diversity Index was used to assess fecal alpha microbial diversity among anemic infants at different stages of infection. (Figure 2). Our analysis shows that there is no significant difference in alpha diversity between non-infected infants (reference) and anemic infants during incubation, early convalescence, and late convalescence stages of infection. Our results suggest that fecal microbial diversity remains stable during active infection and is not significantly impacted by the stages of infection alone.

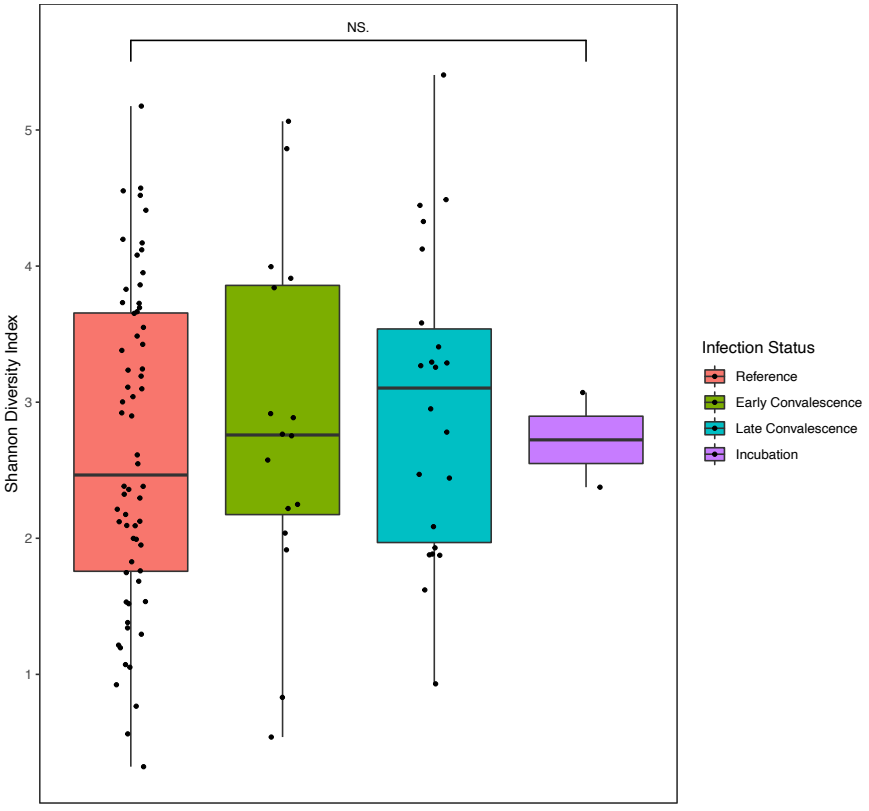

FIG. 2 No significant difference in fecal microbial diversity across anemic patients during different stages of infection. Shannon's diversity index was used to assess fecal alpha diversity of anemic patients during reference (red), early convalescence (green), late convalescence (blue), and incubation (purple). ANOVA and Tukey's post-hoc test showed no significance between any of the groups.

Infected anemic infants on iron supplementations have higher fecal microbial diversity. We assessed fecal microbial diversity between infected anemic infants taking iron supplements and those who did not by using Shannon's Diversity index (Figure 3). Alpha diversity between the two supplementation groups was significantly different, with those on iron supplementation having increased diversity. These results indicate that iron supplementation during active infection within anemic infants leads to greater microbial diversity, while infants who do not take nutrient supplements display reduced microbial diversity.

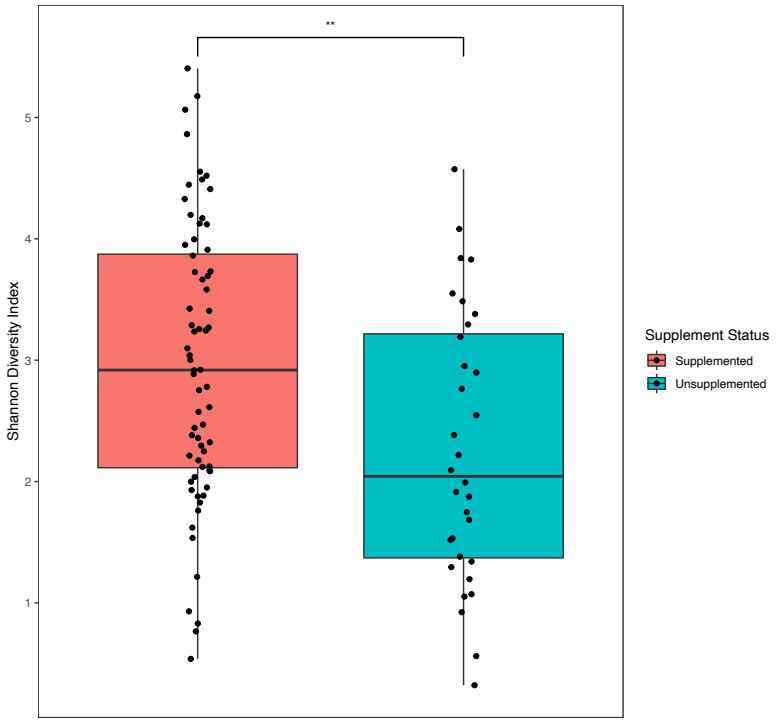

FIG. 3 Significant difference in microbial diversity between supplemented and non-supplemented infected anemic patients. Alpha diversity was measured between supplemented (red) and unsupplemented (blue) infected patients using Shannon's Diversity Index. A Shapiro wilks test was used to assess for normality and a paired t-test ($p = 0.005$) showed that there is a significant difference in microbial diversity between the two groups.

Indicator species analysis reveals inflammatory species for anemic infants with infection and no nutrient supplementation. We conducted indicator species analysis to explore the relationship between different sample groups and indicator species in the context of anemia and infection. Four groups were defined based on the combination of infection status (Reference or Infected) and nutrient supplement intake (Supplement or No supplement). Our results showed a total of 21 significant species ($p < 0.05$), and the complete ISA results are under supplementary Tables S1 and S2. Out of 834 species of bacteria in this subsetted dataset, indicator species analysis identified 9 species for anemic, infected infants and 8 species for anemic, uninfected infants. Our findings showed that anemic, infected infants who did not receive any supplements had higher abundance of indicator species associated with inflammation and pathogenicity and notable indicator species with high relevance are listed in Table 1. In general, anemic infants who have infection and are not taking supplements tend to display more inflammatory and pathogenic indicator genera. There is also a lack of bacteria with probiotic characteristics within these infants. Additionally, within this group the association of pathogenic bacteria is higher than the other groups. However, non-infected anemic infants all show probiotic bacteria, as well as infected infants who take nutrient supplements. In sum, these results highlight the importance of probiotic interventions and nutrient supplementation in enhancing the gut microbiota of anemic infants with infections.

DISCUSSION

Supplementation status within anemic infants does not alter fecal microbial diversity. We first aimed to investigate whether iron supplementation affects fecal microbial diversity in 12-month-old anemic infants. The results of the study indicated that iron supplementation, whether in the form of FeSO4 or micronutrient powders (MNP), did not significantly affect fecal microbial diversity in anemic patients compared to those who did

TABLE. 1 Notable indicator species from indicator species analysis. Indicator species were selected based on relevance after preliminary literature review. Shows comparison group (whether reference or infected, on supplements or off), genus (g) or family (f) of indicator species, species name if available, characteristics of indicator species, association statistic, and p-value. The "association" parameter in this context refers to the strength of the association between a particular species of bacteria and a specific condition (such as anemia or infection). It is typically measured using statistical methods and represented by a statistical value (such as p-value) that indicates the likelihood of the observed association occurring by chance. In this table, the association parameter represents the strength of the association between the indicator species and the corresponding condition, as measured by the indicator value.

Group	Genus	Characteristic	Association	p-value
Reference, Supplement	(g) Bacteroides	Known to metabolize polysaccharides and oligosaccharides, provides nutrients to the host and other gut microbes (27).	0.60	0.045
Reference, No supplement	(g) Bifidobacterium	Among the predominant microorganisms during infancy, beneficial to infant health (27)	0.47	0.01
	(g) Blautia	Anaerobic bacteria with probiotic characteristics (28)	0.37	0.025
Infected, Supplement	(g) Lactobacillus *Lactobacillus mucosae*	Adheres to intestinal mucus and to inhibit pathogens in the gastrointestinal tract (29)	0.80	0.045
Infected, No supplement	(g) Enterococcus	Includes species of opportunistic pathogens (31-32)	0.68	0.025
	(f) Enterobacteriaceae	Some species are observed in infectious disease settings and contribute to gut dysbiosis (35)	0.78	0.035
	(g) Megasphaera *Megasphaera micronuciformis*	Megasphaera micronuciformis DSM 17226 was isolated from human liver abscess (36)	0.53	0.04
	(f) Lachnospiraceae	Some taxa associated with various diseases (37)	0.53	0.03

not take supplements. This finding is inconsistent with previous findings that iron supplementation is associated with alterations in the fecal microbiome, which could influence long-term iron homeostasis (20).

One possible explanation for the lack of significant alterations in microbial diversity following iron supplementation could be due to the short duration of the intervention which lacked the sensitivity to detect subtle changes in the fecal microbiome over time (1-4). Another possible reason that may be attributed to our finding is that the dosage of iron supplement was not documented in the dataset and may not be comparable to other studies. Previous research showed that a high iron dose is usually administered to ensure that sufficient iron is absorbed, despite the presence of inhibitory complementary foods and inflammation-mediated increases in serum hepcidin levels, which is a peptide that hinders iron absorption in the gut (21). Together, our findings have implications that appropriate dosage of iron supplementation can be safely used to treat iron deficiency anemia in infants without causing significant alterations in microbial diversity. However, future studies are required to assess a larger dataset as well as the long-term effects of iron supplementation on gut microbiota composition and function in infants.

Infection within anemic infants does not alter fecal microbial diversity. After investigating the effect of iron supplementation on anemic infants, we aimed to determine the impact of active infection on the fecal microbiota of anemic infants through alpha diversity analysis. Our results suggest that active infection alone is not sufficient to alter the fecal microbiota within anemic infants and that the difference in microbial composition is not significant between infection stages. This is contradictory to literature as previous studies have shown that gut microbial diversity experiences alterations in response to infection (22-24). The absence of change in diversity may be attributed to the resilience of the gut microbiota as it has been shown to resist changes in other variables such as diet, invasion by

new species, and antibiotic administration (24). It is unclear what types of infections were being documented in the metadata and therefore the type of infection may be a confounding variable, in addition to other variables such as diet and nutrient supplementation. It should also be noted that due to the constraints of our dataset, the sample size of anemic infants in the reference stage of infection was small (n = 2) and likely unrepresentative of the incubation cohort. A larger sample would be required to determine if there were any significant differences in diversity between infants within the incubation stage of infection and non-infected infants.

Alpha diversity increases in infected anemic infants following iron supplementation. After our analysis of microbial diversity across the different stages of infection produced insignificant results, we examined the effects of iron supplementation on infected anemic infants. Our results indicate that iron supplementation results in a greater alpha diversity within the fecal microbiota of infected anemic infants. Therefore, it appears that the interplay between active infection and iron supplementation may be important for altering microbial diversity, as neither factor alone showed a significant effect. Previous literature has shown that pathogenic species carry genes that are more efficient in iron metabolism which may allow these pathogenic species to better adapt to low iron environments (25). Further, chronically ill patients have gut microbiota that are low in microbial diversity and have high occurrences of pathogenic species (26). A possible interpretation of our results could be that commensal gut microorganisms within unsupplemented and infected anemic infants are decreased due to competition from pathogenic species and inadequate levels of iron, which have been shown to be essential for bacterial growth (27). It is likely that the observed increase in microbial diversity of anemic infants is due to the restorative effects of iron supplementation on the microbiota. Additionally, we may have been unable to detect any variations in microbial diversity while looking at only infection statuses as it did not account for any confounding variables such as diet or iron supplementation.

Iron supplementation of anemic infants with inflammatory markers may reduce prevalence of pathogenic species in the gut. To explore the significant result between supplementation status for infected anemic infants, we subset the data into four groups and performed indicator species analysis. Analyses of the microbial composition of anemic infants based on if they have infection and whether they take nutrient supplements or not shows that nutrient supplementation for anemic infants has beneficial effects on the gut microbiome. While the sample size of infected infants not on supplements is small (n = 7), limiting the statistical power, the indicator species have relatively higher association statistics and smaller p values which indicate strong association.

Among uninfected infants and supplemented infected infants, we observe probiotic indicator species in genera like *Bacteroides*, *Bifidobacterium*, *Blautia*, and *Lactobacillus*. *Bacteroides* and *Bifidobacterium* have been implicated in being important for immune development and are abundant in the normal infant microbiome (28). *Blautia* has been implicated in being a probiotic genus (29). *Lactobacillus mucosae* can adhere to the intestinal mucus and inhibit pathogens (10). Similarly, strain DPC 6426 can influence the immune system and have cardio-protective properties (31). Indicator species analysis reveals that both anemic infants who do not have infection and anemic infants who are infected but take nutrient supplements have a relatively probiotic and protective microbiome.

However, infected infants who do not take supplements tend to have pathogenic indicator species. For instance, *Enterococcus* bacteria include commensal species and are often part of the core microbiome, but they are also known to cause infections (32). Additionally, *Enterococcus faecalis* can scavenge for iron, contributing to its virulence (33). In iron poor environments like the guts of anemic infants, pathogenic species with efficient iron uptake systems may outcompete other commensal bacteria, explaining the prevalence of opportunistic pathogens in infected anemic infants not taking supplements. Indeed, a study on mice using an iron-rich and iron-poor diet showed that pathogenic species are less affected by a low iron diet as they are able to scavenge iron more effectively (34). However, *E.faecalis* may also be probiotic as it has been shown to regulate the infant immune system, maintaining immune balance (35). *Enterobacteriaceae* are known to play a role in encouraging microbial dysbiosis and gut inflammation (36). *Megasphaera micronuciformis* is a potential pathogen as it was isolated from liver abscess, and Lachnospiraceae have been associated with various

human diseases, although they are also known to produce beneficial metabolic products (37-38). While there are possible probiotic characteristics of the indicator species seen in anemic infected infants not taking supplements, they display characteristics of opportunistic pathogens and pro-inflammatory species to an extent not seen in the other three cohorts.

The analysis conducted indicates that unsupplemented infected infants show significantly lower alpha diversity, providing support for the presence of an inflammatory gut microbiome in these infants. A less diverse microbiome may result in a more inflammatory gut (39). As mentioned before, iron deficiency may lead to pathogenic bacteria becoming more prominent and outcompeting other species which is reflected in our ISA results as well as comparison of alpha diversities. Collectively, these results show that nutrient supplementation of infected anemic infants results in probiotic indicator species and prevents the appearance of pathogenic bacterial taxa.

Limitations One limitation was the small sample size of anemic patients who were not on supplements (n=11), which may not accurately represent the entire population being studied. Similarly, we were unable to determine whether infants during the incubation stage of infection have variations in microbial diversity in comparison to non-infected infants due to our small sample size. Linear regression with diversity as a response variable and age and infection status as predictor variables indicated that they were strong predictors of diversity ($p = 1.009e-06$). A similar result was seen for age and supplementation as a predictor of diversity ($p = 5.493e-07$). Due to the constraints of our sample size and therefore statistical power, we were unable to subset the infants by 6 months and 12 months and a larger sample size would be required to further test for any significant effect of age on diversity. Another limitation was the use of CRP to infer infection status, which may not be the most ideal method. This is due to CRP being an inflammation marker that can be altered by various factors such as diet, age, autoimmune disease, viral infection, and bacterial infection. Therefore, it may not be specific enough to identify infants who are in the incubation stage of infection and may have variations in microbial diversity compared to non-infected infants.

Conclusions Our study aimed to assess the effects of iron supplementation and infection on microbial diversity within anemic infants. We found that supplementation and infection alone do not have any significant effect on the fecal diversity of anemic infants. Further analysis of infected anemic infants on supplementation showed that the cohort with iron supplementation have greater alpha diversity than the cohort without supplementation. Additional analysis with indicator species indicates that iron supplementation for infected anemic infants does not affect commensal bacteria and reduces the occurrence of pathogenic species. Our findings from this study show that iron supplementation should be administered to improve gut microbial health within anemic infants, especially infants that reside in lower- and middle-income countries. Further, iron supplementation does not negatively affect microbial health by impacting commensal species. These results may have important implications on how to provide better standardized care for infants with IDA.

Future Directions A major limitation of our study was the small sample size of anemic patients who were not on supplements. As the exact mechanisms as to which iron supplementation can reduce the prevalence of pathogenic species is not well elucidated, future studies may consider repeating this investigation using a larger and well-distributed sample size (2-6). For instance, collecting more samples from infected anemic infants who do not take supplements should be a priority as well as collecting samples from countries where iron-rich foods are an important part of the cultures' diet, to see how cultural diet can influence the relationship between anemia, iron supplementation, and the prevalence of pathogenic species. Furthermore, exploring the potential role of other dietary factors or interventions in modifying the gut microbiota in anemic patients could also be an interesting avenue of research.

Additionally, future studies should aim to collect more samples from infants in the incubation period of infection and perform further functional analyses on the identified pathogenic species. For instance, this could involve obtaining datasets that incorporate single-

cell RNA sequencing to obtain gene expression signatures associated with pathogenic infiltration. This will allow for analyzing differential gene expression between samples with infection and those without using DESeq2 analysis; hence, providing us with comparisons with the proportion of reads that align with pathogenic or beneficial indicator species. Future studies can also use PICRUSt2 to predict functional outcomes and explore how nutrient supplementation affects protein catabolism by intestinal bacteria.

Another notable limitation in our study was the use of CRP to infer infection status among infants with IDA. Although CRP is an extensively studied marker and has been used to distinguish bacterial from non-bacterial infections, its positive predictive value is insufficient to be used on its own (40). One reason for this is that CRP levels can be elevated in response to non-infectious causes of inflammation, such as trauma, surgery, or autoimmune diseases. Furthermore, some infections may not result in a significant increase in CRP levels, in particular viral infections such as viral gastroenteritis. This is because viral infections often do not produce as much tissue damage and inflammation as bacterial infections (40). Therefore, while CRP can be a useful indicator of inflammation in general, it is not a specific or sensitive enough marker to reliably determine infection status on its own. Other diagnostic tests, such as blood cultures or PCR (polymerase chain reaction) testing, are often needed to confirm the presence of infection. One future direction for our research could be to explore studies that consider the aforementioned diagnostic tests in addition to CRP levels or studies that make use of more specific biomarkers for identifying infection status in infants. For example, confirmation of specific pathogenic bacteria or the use of cytokines and other immune system markers could provide a more accurate and specific indication of infection status, as these functional features can help us directly link immune response to infection (40-41). Another possible direction could be to combine multiple biomarkers to improve the accuracy of identifying infection status. As such, future experiments can include using a combination of CRP, cytokines, and other immune system markers to create a more comprehensive picture of infection status in infants and enable more in-depth analysis (41).

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CONTRIBUTIONS

Ryan Hong worked on the abstract, introduction, future directions, compiled references, and overall facilitation of the paper.

Griff Wong worked on methods, results, and discussion for figure 2 and 3, conclusion, and assisted with limitations.

Jason Zhao worked on data processing and exporting with QIIME 2 and methods, results, and discussion for indicator species analysis.

Tian Zhao worked on methods, results, and discussion for figure 1 along with limitations, and assisted with the abstract.

All co-authors contributed to data analysis and summarizing conclusions for the data. All co-authors contributed to the editing and revision of the manuscript in its entirety, reviewing and providing feedback. Co-authorship credit should be considered equal for all authors.

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