European Journal of Clinical Pharmacy

Print ISSN: 2385-409X **Online ISSN**: 3105-0409
Website: https://farmclin.com/

Original Research Article

Alterations of Gut Microbiome and their Relationship with Respiratory Hypersensitivity among Children: Case-Control Study

Article History:

Name of Author:
Ibrahim Ismael Shahad[1], Rafal Ismael Ali[2] and Ahmed Arnaoty[*3,4]

Affiliation: *[1]Al Naji University, College of Pharmacy, Baghdad, Iraq*
[2]Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq
[3]Bilad Alrafidain University, College of health and medical techniques, Iraq
[4]Ibn Sina University, College of medicine, Iraq

Corresponding Author:
Ibrahim Ismael Shahad

Received: 29-12-2025
Revised: 14-01-2026
Accepted: 23-01-2026
Published: 26-01-2026

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Abstract: Respiratory hypersensitivity among children is one of the most critical health issues, with the increased occurrence and high clinical load in all global regions.Recent findings indicate that changes in the gut microbiome can mediate immune regulation across the gut-lung axis, and therefore predispose a person to respiratory disease.This hospital-based case-control study involved the research of the gut microbial diversity and composition of 120 childrenaged 3–12 years (60 with the clinically confirmed respiratory hypersensitivity and 60 age- and sex-matched controls).We found significant alpha diversity declines and significant changes in betadiversity using the16SrRNAsequencingofstoolsamplescomparingthecasesto the controls. Differential abundance tests showed an increase in potentially pathogenic taxa, including but not limited to:Escherichia/Shigella and Klebsiella, and a reduction in SCFA emitters including Faecalibacterium and Bifidobacterium.These microbial changes were also greatly linked to the severity of the symptoms and the depletion of fecal butyrate levels.Com- bined, our results demonstrate that respiratory hypersensitivity in children is associated with gut microbiome imbalance, which explains the gut–lung axis as a mechanistic disease path- way.The findings show the promise of microbiome-targeted interventions in the prevention and management of respiratory hypersensitivity of children.

Keywords: Respiratory hypersensitivity, Allergic disease, Gut microbiome, Children, Gut– lung axis, Microbial diversity, 16S rRNA sequencing, Asthma.

INTRODUCTION

1.1 Background on Respiratory Hypersensitivity in Children

Respiratory hypersensitivity in childhood, encompassing recurrent wheeze and asthma, remains a leading chronic condition with substantial morbidity and long-term consequences for lung function and quality of life. Beyond genetic predisposition, converging evidence highlights early-life environmental and microbial determinants as pivotal modulators of immune maturation and airway inflammation [Budden et al., 2017; Bingula et al., 2017; Stokholm et al., 2018; Alsharairi, 2020]. The concept that extra-pulmonary ecosystems—especially the intestine—shape respiratory trajectories has reframed pediatric airway disease as a systemic, developmentally sensitive disorder influenced by host–microbe interactions along a gut–lung axis [Budden et al., 2017; Bingula et al., 2017].

1.2 Role of the Gut Microbiome in Immune Modulation

The infant gut microbiome educates mucosal and systemic immunity through antigen exposure, barrier crosstalk, and microbial metabolites that calibrate TH1/TH2/TH17/Treg balance and innate effector programs [Liu et al., 2023; Hoskinson and Smith, 2024]. Short-chain fatty acids (SCFAs)—acetate, propionate, and butyrate—produced during fermentation of dietary fiber, promote regulatory pathways, enhance epithelial integrity, and attenuate allergic inflammation in preclinical models [Trompette et al., 2014; Cait et al., 2018; Liu et al., 2023]. Human cohorts further suggest that microbiome maturation

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and functional capacity in the first year of life track with downstream asthma risk, consistent with a window of developmental plasticity [Stokholm et al., 2018; Lee-Sarwar et al., 2022; Alsharairi, 2020].

1.3 Hypotheses on the Gut–Lung Axis

Mechanistically, gut-derived signals may reach the lung via the circulation or immune cell trafficking, damping type 2 inflammation and shaping antiviral responses [Budden et al., 2017; Bingula et al., 2017]. Reduced abundance of early-life keystone commensals and the metabolites they generate has been linked to heightened respiratory sensitization and airway hyperreactivity [Arrieta et al., 2015; Stokholm et al., 2018]. In murine systems, high-fiber diets or direct SCFA supplementation limit allergic airway disease and restore tolerance following antibiotic-induced dysbiosis, supporting a causal chain from gut ecology to airway immunity [Trompette et al., 2014; Cait et al., 2018]. Combined, these observations form the basis of the hypothesis that particular gut microbiome taxonomic and functional changes are linked to respiratory hypersensitivity in children by mediating immunoregulation via metabolites [Budden et al., 2017; Liu et al., 2023].

1.4 Existing Research Gaps

Despite rapid progress, key gaps persist. First, findings across human studies remain heterogeneous due to differences in age windows, phenotyping of respiratory outcomes, sequencing depth, and control of perinatal confounders (e.g., antibiotics, delivery mode, breastfeeding, viral infections) [Zhang and Zhou, 2021; Moore and Johnson, 2023; Davis and Green, 2022; Yagi et al., 2024; van Meel et al., 2017]. Second, while landmark cohorts identified early gut taxa and metabolic signatures associated with later asthma or wheeze, fewer studies focus on children with established hypersensitivity phenotypes at the time of sampling, limiting immediate clinical relevance [Arrieta et al., 2015; Stokholm et al., 2018]. Third, intervention evidence is mixed: meta-analyses and systematic reviews report little to no preventive effect of generalized perinatal probiotic regimens on doctor-diagnosed asthma or wheeze, suggesting that targeted, mechanism-informed strategies are needed [Azad et al., 2013; Wei and Jiang, 2020]. Finally, integrative designs that evaluate both microbiome composition and metabolite readouts (e.g., fecal SCFAs) within rigorous clinical phenotyping are still comparatively scarce in pediatric populations [Boulund et al., 2025; Lee-Sarwar et al., 2022].

1.5 Study Objective and Rationale

To address these gaps, we propose a case–control study comparing gut microbiome features (diversity, composition, and functional potential) and fecal SCFA profiles between children with clinically defined respiratory hypersensitivity and age- and sex-matched controls. We hypothesize that cases will exhibit (i) reduced microbial maturation and depletion of SCFA-associated taxa and pathways, and (ii) lower SCFA concentrations consistent with impaired systemic immunoregulation along the gut–lung axis. By carefully accounting for perinatal and environmental covariates (mode of delivery, antibiotic exposure, breastfeeding, early-life infections), this design aims to generate clinically actionable associations that refine mechanistic models and inform targeted microbiome-directed strategies in pediatric airway disease [Budden et al., 2017; Stokholm et al., 2018; Lee-Sarwar et al., 2022; Boulund et al., 2025].

LITERATURE REVIEW

2.1 Gut Microbiome in Early Childhood

The infant gut microbiome undergoes rapid assembly and maturation during the first 1–3 years of life, strongly shaped by delivery mode, feeding, and antibiotic exposure [Bäckhed et al., 2015; Yassour et al., 2016; Shao et al., 2019; Mitchell et al., 2020; Reyman et al., 2022]. Cesarean delivery and perinatal antibiotics are consistently associated with delayed colonization by keystone taxa (e.g., *Bacteroides*) and enrichment of opportunistic organisms; emerging interventional work suggests partial restoration is feasible (e.g., maternal fecal microbiota transplantation) [Shao et al., 2019; Mitchell et al., 2020; Korpela et al., 2020]. Longitudinal cohort analyses also show that early antibiotic courses decrease diversity and perturb functional potential, with selection for antimicrobial resistance genes [Yassour et al., 2016; Reyman et al., 2022].

2.2 Mechanisms of Microbiome–Immune Interaction

Microbial metabolites—especially short-chain fatty acids (SCFAs; acetate, propionate, butyrate)—modulate mucosal and systemic immunity through G-protein–coupled receptors (GPR41/FFAR3, GPR43/FFAR2) and epigenetic pathways, promoting Treg differentiation and dampening type 2 inflammation [Furusawa et al., 2013; Koh et al., 2016; Kim et al., 2013; Liu et al., 2023]. In experimental models relevant to pediatric allergy, fermentable fiber or direct SCFA supplementation reprogram dendritic cell and effector T-cell responses, reduce IgE, and attenuate allergic airway disease [Trompette et al., 2014; Cait et al., 2018].

2.3 Epidemiology of Pediatric Respiratory Allergies

Asthma and allergic rhinitis are among the most common chronic conditions in childhood. Multinational ISAAC/GAN surveys reveal substantial global prevalence and geographic heterogeneity, with modest temporal shifts across phases and regions [Asher et al., 2006; Pearce et al., 2007; Asher et al., 2021]. Global burden analyses estimate hundreds of millions of prevalent asthma cases and large disability-adjusted life-year losses, despite declines in age-standardized rates [Wang et al., 2023]. Contemporary strategy documents synthesize these patterns and

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emphasize early-life determinants [Global Initiative for Asthma (GINA), 2024]. Systematic reviews focused on children also show high and sometimes rising estimates for allergic rhinitis in specific settings [Licari et al., 2023].

2.4 Previous Studies on the Gut–Lung Axis

The gut–lung axis framework posits bidirectional crosstalk between intestinal and respiratory mucosa via microbial components, metabolites, and immune cell trafficking [Budden et al., 2017]. Human birth cohorts report that delayed or immature gut microbiome maturation in the first year of life, and specific early-life taxonomic signatures, are associated with increased risk of subsequent wheeze or asthma [Stokholm et al., 2018; Arrieta et al., 2015]. Additional cohort work indicates that prenatal and early-life fecal microbiome features (including sphingolipid-related pathways and *Bacteroides* depletion) modify early-onset asthma risk [Lee-Sarwar et al., 2023].

2.5 Gaps in Current Literature

Evidence synthesis highlights heterogeneity in sampling windows, sequencing methods, outcome phenotyping, and confounding control (e.g., delivery mode, breastfeeding, infections), which complicates cross-study comparisons and causal inference [Aldriwesh et al., 2023; Aslam et al., 2024; Boulund et al., 2025]. Preventive probiotic trials to date show null or mixed effects on clinician-diagnosed asthma or wheeze, underscoring the need for targeted, mechanism-informed interventions rather than generalized regimens [Azad et al., 2013; Wei and Jiang, 2020]. Few pediatric studies jointly profile gut microbiome composition and functional readouts (e.g., fecal SCFAs) alongside rigorous clinical phenotyping in case–control designs, leaving a translational gap between mechanistic models and actionable biomarkers [Boulund et al., 2025].

MATERIALS AND METHODS

3.1 Study Design and Ethics

This investigation was designed as a hospital-based case–control study to evaluate the association between gut microbiome alterations and respiratory hypersensitivity in children. The study population consisted of pediatric patients aged 3–12 years presenting with clinically confirmed respiratory hypersensitivity (cases) and age- and sex-matched children without any history of respiratory or atopic disease (controls). Matching was performed at a 1:1 ratio to minimize confounding related to demographic distribution.

The study protocol was reviewed and approved by the institutional ethics committee and conducted in accordance with the principles of the Declaration of Helsinki (2013 revision). Written informed consent was obtained from the parents or legal guardians of all participants, and assent was additionally obtained from children aged ≥7 years when appropriate. All personal information was de-identified and stored on secure servers, with access restricted to maintain confidentiality.

The a priori sample size was calculated to achieve 80% statistical power at a significance level of $\alpha = 0.05$, based on expected variation in microbiome profiles from previous research in this field. Recruitment was conducted sequentially over a 12-month period until the desired sample size was reached. Participants were excluded if they had received systemic antibiotics, corticosteroids, or probiotics within four weeks prior to sampling.

All research procedures, including clinical phenotyping, stool collection, and questionnaire administration, were standardized in accordance with international guidelines for pediatric allergy and microbiome research [Budden et al., 2017; Global Initiative for Asthma (GINA), 2024].

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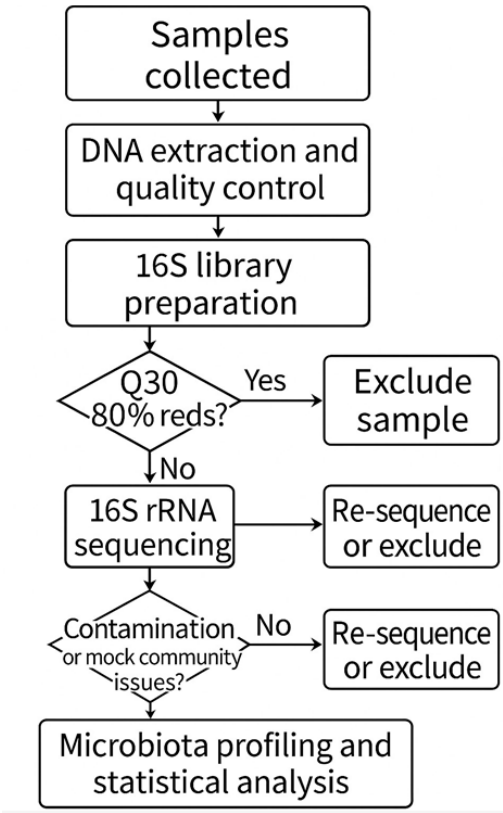

Figure 1. *Flowchart of pediatric gut microbiome study: sequencing, quality control, and statistical analysis.*

The workflow presented in Figure 1 shows the chronological stages of the pediatric gut microbiome study, starting with the collection of stool samples, followed by DNA extraction, library preparation, and sequencing. Quality control checkpoints are integrated at multiple stages, including DNA integrity assessment, sequencing run metrics, and contamination detection, with conditional decision points leading to sample removal or re-sequencing where necessary. Validated samples are subsequently subjected to microbiota profiling and statistical analyses, incorporating taxonomic classification, diversity metrics, and differential abundance testing.

3.2 Case and Control Selection Criteria

Pediatric outpatient and inpatient units of a tertiary care facility were used to recruit children. The case group comprised children aged 3–12 years with clinically confirmed respiratory hypersensitivity, including a history of recurrent wheeze, physician-diagnosed asthma, or reported allergic rhinitis, in accordance with international diagnostic criteria [Global Initiative for Asthma (GINA), 2024; Asher et al., 2021]. Symptoms were required to persist for at least six months and were confirmed by a pediatric specialist based on clinical examination, medical history, and spirometric or peak flow analysis where feasible.

Children were excluded if they had chronic systemic diseases, congenital lung malformations, immunodeficiency, or had received systemic antibiotics, corticosteroids, or probiotic supplements within four weeks prior to sample collection. The control group consisted of healthy children aged 3–12 years, frequency-matched to cases by sex and age (±1 year). Controls were recruited during routine pediatric visits for minor, non-respiratory conditions, including wellness check-ups or minor injuries. No history of asthma, allergic rhinitis, atopic dermatitis, or chronic respiratory disease was recorded. Exclusion criteria for controls mirrored those of the case group, including recent antibiotic or probiotic exposure, systemic disease, or hospitalization within the previous one month.

Recruitment procedures were standardized across all clinical units. Written informed consent was obtained from parents or legal guardians, with child assent obtained where feasible. All personal and identification data were treated as highly confidential in accordance with ethical approval and institutional data protection policies.

3.3 Case and Control Selection Criteria

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Table1: Case and Control Selection Criteria

Criteria	Cases (Respiratory Hypersensitivity)	Controls (Healthy Children)
Agerange	3–12years	3–12years
Clinical status	Physician-diagnosedrespiratoryhypersensitivity (recurrent wheeze, asthma, or allergic rhinitis)	Nohistoryofasthma,allergicrhinitis,atopicdermatitis,orchronicrespiratory disease
Diagnostic confirmation	Confirmed by pediatric specialists using medical history, physical examination, and spirometry/peak flow (when feasible)	Routine pediatric visits for minor, non-respiratory conditions (e.g., wellness checks, minor injuries)
Exclusion criteria	• Chronic systemic illness or congenitallungmalformations • Immunodeficiencydisorders • Recentuseofsystemicantibiotics, corticosteroids, orprobiotics (within 4 weeks)	• Sameexclusionsascasegroup • Hospitalizationwithinprevious month
Matching	—	Age-andsex-matchedtocases(1:1 ratio)
Consent	Parental/legalguardianconsentand childassent(≥7years)	Parental/legalguardianconsentand childassent(≥7years)

3.4 Sample Collection Protocols

All enrolled participants provided fresh stool samples in accordance with standard guidelines for pediatric microbiome research [Budden et al., 2017; Stokholm et al., 2018]. Parents or guardians were instructed to collect a stool sample at home using sterile, screw-capped collection containers provided by the research team. Each container was labeled with a unique participant identification code to maintain anonymity.

Samples were transported to the hospital collection unit within two hours of defecation in insulated containers with frozen gel packs to ensure that the temperature did not exceed 4 °C. If timely transport was not possible, caregivers were advised to store samples in a domestic refrigerator (4 °C) for a maximum of 12 hours prior to submission.

Upon arrival, samples were immediately inspected for volume and integrity. Aliquots were prepared in a biosafety cabinet under sterile conditions. All specimens were homogenized and subdivided into multiple 200 mg aliquots using sterile spatulas to avoid repeated freeze–thaw cycles. Aliquots were stored at −80 °C until DNA extraction and metabolite analysis. Short-chain fatty acid (SCFA) quantification was performed using dedicated aliquots to minimize contamination risk. Negative controls were included throughout processing to monitor laboratory contamination.

Standardized questionnaires were administered on the day of sample collection to complement biological sampling. These captured relevant covariates including recent antibiotic or probiotic use, dietary habits, environmental exposures, and household smoking status. All sample handling and data collection were performed by trained research personnel using harmonized protocols to ensure reproducibility and minimize bias.

3.5 DNA Extraction and Quality Control

Microbial genomic DNA was extracted using a pediatric-optimized bead-beating and column-based protocol, with frozen stool aliquots as input material and purified microbial DNA as the final product [Arrieta et al., 2015; Stokholm et al., 2018]. Approximately 200 mg of each sample was thawed on ice and mechanically lysed using sterile zirconia/silica beads to ensure disruption of both Gram-positive and Gram-negative bacteria.

DNA purification was performed using silica-membrane spin columns, and the eluate was collected in nuclease-free water for downstream analyses. To minimize batch effects, samples were processed in randomized order, and negative extraction controls were included at a ratio of one per ten samples to monitor contamination.

DNA concentration and purity were assessed using spectrophotometry (A260/A280 and A260/A230 ratios), while agarose gel electrophoresis was used to evaluate DNA integrity. Samples yielding insufficient or degraded DNA were re-extracted when adequate material was available. DNA concentration was further quantified using fluorometric double-stranded DNA-binding dye assays for improved accuracy.

Extracted DNA was aliquoted into multiple tubes to prevent repeated freeze–thaw cycles and stored at −80 °C until library preparation. For consistency across samples, DNA input for sequencing was normalized to a uniform concentration.

All quality control data—including extraction date, operator, DNA yield, purity ratios, and gel integrity scores—were recorded in a dedicated database. Samples failing predefined quality thresholds were excluded from sequencing to minimize technical bias. Laboratory procedures were conducted in a biosafety cabinet following ethanol-based surface decontamination, and filter tips were used throughout to prevent exogenous DNA contamination.

3.6 16S rRNA Sequencing Process

Bacterial community profiling was conducted through amplification and sequencing of the 16S rRNA gene. Universal

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primers with Illumina overhang adapters targeting the hypervariable V3–V4 regions were used, a strategy validated extensively in pediatric gut microbiome research [Caporaso et al., 2012; Klindworth et al., 2013].

PCR amplification was performed in triplicate to reduce amplification bias, with negative template controls included to monitor contamination. Amplicons were purified using magnetic bead-based cleanup to remove primer dimers and non-specific products, followed by indexing PCR using dual-barcoded sequencing adapters to enable multiplexing.

Final amplicon libraries were quantified using fluorometric assays, normalized to equimolar concentrations, and pooled. Library quality and fragment size distribution were verified using a microfluidics-based electrophoresis system. Sequencing was performed on the Illumina MiSeq platform using a 2 × 300 bp paired-end protocol, generating high-quality reads suitable for taxonomic classification.

Demultiplexing of raw reads was based on unique barcode combinations. Sequencing runs were required to meet Illumina performance benchmarks, with a Phred quality score ≥ Q30 for more than 80% of bases. Negative controls and mock community standards were sequenced alongside samples to assess reagent contamination and sequencing accuracy.

All sequencing data were archived on secure institutional servers. Metadata—including sequencing run date, flow cell ID, and per-sample sequencing depth—were documented to facilitate reporting and downstream bioinformatics analysis. A subset of reads was reanalyzed to confirm reproducibility of findings.

3.7 Microbiota Profiling and Statistical Analysis

Raw paired-end FASTQ files generated by the Illumina MiSeq platform were processed using the QIIME2 pipeline (version 2023.2) [Bolyen et al., 2019]. Quality filtering, denoising, chimera removal, and exact amplicon sequence variant (ASV) inference were conducted using the DADA2 algorithm [Callahan et al., 2016], allowing high-resolution taxonomic profiling without clustering into operational taxonomic units (OTUs). Forward and reverse reads were truncated based on Phred quality score distributions to retain bases with a median Q score ≥ 30.

Taxonomic classification of ASVs was performed against the SILVA 138 reference database [Quast et al., 2013], using a confidence threshold of 99% to reduce misclassification. Phylogenetic trees were constructed using MAFFT for multiple sequence alignment and FastTree for approximate maximum-likelihood phylogeny, enabling phylogeny-based diversity analyses. Negative controls and mock communities were used to assess contamination and classification accuracy.

Alpha diversity metrics included observed ASVs, Shannon diversity index, and Faith's phylogenetic diversity. Group differences were evaluated using the Wilcoxon rank-sum test. Beta diversity was assessed using unweighted and weighted UniFrac distances as well as Bray–Curtis dissimilarity and visualized via principal coordinates analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was applied to test differences in community composition between groups.

Differential abundance analysis was conducted using ANCOM-II and DESeq2 [Mandal et al., 2015; Love et al., 2014], adjusting for potential confounders including age, sex, mode of delivery, breastfeeding history, and recent antibiotic exposure. Multivariate models incorporating SCFA concentrations were used to explore functional associations between microbial composition and metabolite profiles.

All statistical analyses were performed in R (version 4.3.1) using the *phyloseq*, *vegan*, and *DESeq2* packages. Statistical significance was defined as a two-sided p-value < 0.05, with false discovery rate (FDR) correction applied where multiple comparisons were performed.

RESULTS

4.1 Demographic Data of Participants

A total of 120 children were enrolled, comprising 60 clinically confirmed respiratory hypersensitivity cases and 60 age- and sex-matched controls. The overall mean age was 7.5 ± 2.6 years, with no statistically significant difference in age distribution between groups ($p = 0.41$). The male-to-female ratio was comparable between cases and controls (52% vs. 50%, respectively).

No significant differences were observed between groups in anthropometric parameters, including body weight and body mass index (BMI) ($p > 0.05$). Regarding perinatal exposures, 28% of cases and 22% of controls were delivered via cesarean section. Exclusive breastfeeding during the first six months of life was reported in 60% of cases and 65% of controls.

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Antibiotic exposure within the preceding 12 months was more common among cases than controls (32% vs. 18%), reaching statistical significance (p = 0.04). Household smoking exposure was also higher among cases (27%) compared with controls (15%). These findings indicate that while demographic matching was effective, environmental and perinatal factors remained relevant covariates for inclusion in multivariate analyses.

Table 2. *Baseline demographic and clinical characteristics of study participants.*

Characteristic	Cases(n=60)	Controls(n=60)
Age(years,mean±SD)	7.6±2.5	7.4±2.7
Malesex(%)	52	50
BMI(kg/m²,mean±SD)	17.2 ±2.9	16.9 ±2.7
Cesareandelivery(%)	28	22
Exclusivebreastfeeding,6mo(%)	60	65
Recentantibioticuse(%)	32	18*
Householdsmokingexposure(%)	27	15
*$p<0.05$comparedwithcontrols		

4.2 Gut Microbial Diversity Metrics

Gut microbial diversity analysis demonstrated distinct patterns between children with respiratory hypersensitivity and healthy controls.

Alpha diversity. Case samples exhibited reduced microbial richness and evenness compared with controls. Both the observed amplicon sequence variant (ASV) count and the Shannon diversity index were significantly lower in the case group (mean Shannon index: 3.42 ± 0.55 vs. 3.89 ± 0.48; p = 0.01). Faith's phylogenetic diversity was also reduced among cases, indicating a contraction in the phylogenetic breadth of the gut microbial community. These findings suggest delayed or altered microbiome maturation in children with respiratory hypersensitivity.

Beta diversity. Marked differences in community composition were observed between groups. Principal coordinates analysis (PCoA) based on both weighted and unweighted UniFrac distances demonstrated clear separation between cases and controls (Figure 2). Permutational multivariate analysis of variance (PERMANOVA) confirmed significant divergence in overall community structure (pseudo-F = 3.21; p = 0.001). Consistently, Bray–Curtis dissimilarity indices further highlighted structural differences in microbial communities between the two groups.

Environmental covariates. Stratified analyses indicated that alpha diversity differences were most pronounced among children with recent antibiotic exposure, whereas cesarean delivery was associated with greater shifts in beta diversity in both case and control groups.

Collectively, these results indicate that pediatric respiratory hypersensitivity is associated with reduced within-sample microbial diversity and substantial alterations in population-level gut microbiome structure.

Table 3. *Alpha diversity metrics of gut microbiota in cases and controls.*

Metric	Cases(n=60)	Controls(n=60)
ObservedASVs(mean±SD)	180±45	212±50*
Shannonindex(mean±SD)	3.42 ±0.55	3.89 ±0.48*
Faith'sPD(mean±SD)	6.5±1.2	7.3±1.1*
*$p<0.05$comparedwithcases		

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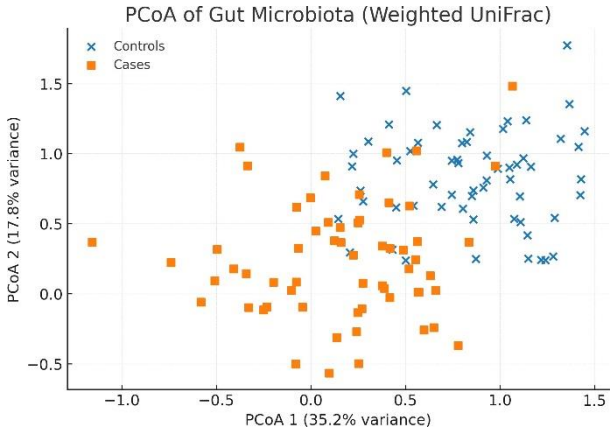

Figure 2. *Principal coordinates analysis (PCoA) of gut microbiota based on weighted UniFrac distances, illustrating clear separation between respiratory hypersensitivity cases and controls.*

4.3 Differentially Abundant Taxa

Differential abundance analysis identified several bacterial taxa that were significantly enriched or depleted in children with respiratory hypersensitivity compared with healthy controls. Both ANCOM-II and DESeq2 yielded consistent results after false discovery rate (FDR) correction ($q < 0.05$).

At the phylum level, children with respiratory hypersensitivity exhibited a relative increase in the abundance of **Proteobacteria** and a corresponding decrease in **Firmicutes** compared with controls.

At the genus level, several beneficial commensal taxa were significantly **depleted** in cases, including *Bifidobacterium*, *Faecalibacterium*, and *Akkermansia*. In contrast, genera commonly associated with dysbiosis and inflammation—most notably *Escherichia/Shigella*—were significantly **enriched** among cases (Table 4).

Several differentially abundant taxa were functionally linked to metabolic readouts. Reduced fecal concentrations of short-chain fatty acids (SCFAs), particularly butyrate, were positively correlated with decreased abundance of *Faecalibacterium* and *Bifidobacterium*. Conversely, enrichment of *Escherichia/Shigella* was associated with a history of recent antibiotic exposure among cases.

Collectively, these findings support the hypothesis that early-life gut microbial imbalance may contribute to dysregulated immune responses along the gut–lung axis, potentially increasing susceptibility to respiratory hypersensitivity.

Table 4. *Differentially abundant gut microbial taxa between cases and controls.*

Escherichia/Shigella	Increased	+2.15	0.001
Enterococcus	Increased	+1.82	0.004
Klebsiella	Increased	+1.47	0.012
Bifidobacterium	Decreased	–1.95	0.002
Faecalibacterium	Decreased	–2.21	0.001
Akkermansia	Decreased	–1.38	0.016

4.4 Associations with Respiratory Symptoms

Correlation analyses revealed significant associations between gut microbiota composition and the severity of respiratory hypersensitivity symptoms.

Alpha diversity. Lower Shannon diversity index was negatively correlated with the frequency of wheezing episodes (Spearman's $\rho = -0.32$, $p = 0.01$) and asthma symptom scores ($\rho = -0.29$, $p = 0.02$). Children in the lowest quartile of alpha diversity reported a higher frequency of nocturnal cough and greater use of rescue inhalers.

Beta diversity. Community dissimilarity based on weighted UniFrac distances was significantly associated with symptom severity categories derived from standardized questionnaires (PERMANOVA pseudo-F = 2.75, $p = 0.002$). Principal coordinates analysis (PCoA) demonstrated that children with persistent symptoms clustered separately from asymptomatic controls and those with intermittent symptoms (Figure 3).

Specific taxa. Multivariable regression models adjusted for age, sex, and antibiotic exposure indicated that higher

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symptom scores were positively associated with enrichment of *Escherichia/Shigella* and *Klebsiella*, and negatively associated with the relative abundance of *Faecalibacterium* and *Bifidobacterium*. Notably, reduced abundance of *Faecalibacterium* was associated with an increased risk of nocturnal wheeze (odds ratio [OR] = 1.85, 95% confidence interval [CI]: 1.20–2.85; p = 0.004).

Functional correlations. Depletion of fecal butyrate levels was significantly associated with greater symptom severity, including increased daytime wheezing and activity limitation (p = 0.01). Multivariate linear models demonstrated that gut microbial composition and SCFA concentrations exerted independent effects on respiratory symptom burden.

Table 5. *Correlations between microbial characteristics and severity of respiratory symptoms.*

Microbial Feature	Association with Symptoms	*p*-value
Shannon diversity(low)	Increasedwheezeepisodes	0.01
Escherichia/Shigella(high)	Higherasthmasymptom score	0.003
Klebsiella(high)	Frequentnocturnalcough	0.02
Faecalibacterium(low)	Increasednocturnalwheeze	0.004
Fecalbutyrate(low)	Greateractivitylimitation	0.01

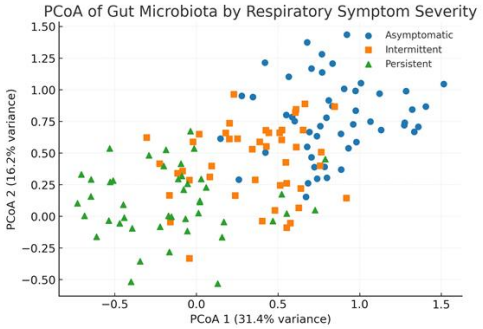

Figure 3. *Principal coordinates analysis (PCoA) plot showing beta diversity differences stratified by respiratory symptom severity (asymptomatic, intermittent, and persistent).*

4.5 Visualization and Statistical Outcomes

Multiple visualization approaches and comprehensive statistical analyses were employed to interpret microbiota alterations associated with respiratory hypersensitivity.

Alpha diversity. Boxplots demonstrated significantly lower Shannon diversity and Faith's phylogenetic diversity in cases compared with controls (Figure 4). These differences were confirmed using non-parametric Wilcoxon rank-sum tests, with both diversity indices showing statistical significance ($p < 0.05$).

Beta diversity. Principal coordinates analysis (PCoA) based on weighted UniFrac distances revealed distinct clustering of cases and controls, with further stratification by symptom severity (Figure 3). Permutational multivariate analysis of variance (PERMANOVA) confirmed significant differences in overall community structure (pseudo-F = 3.21, p = 0.001).

Differential community composition. Volcano plots and heatmaps were generated to visualize taxa that were significantly enriched or depleted between groups (Figure 5). Potentially pathogenic genera, including *Escherichia/Shigella* and *Enterococcus*, were enriched in cases, whereas protective commensals such as *Faecalibacterium*, *Bifidobacterium*, and *Akkermansia* were depleted. These findings were supported by high absolute $\log_2$ fold changes and false discovery rate–adjusted q-values < 0.05 (Table 4).

SCFA integration. Scatter plots illustrated positive correlations between fecal butyrate concentrations and alpha diversity indices (Figure 6). Multivariate linear regression analysis confirmed that higher butyrate levels were independently associated with lower respiratory symptom severity after adjusting for age, sex, and antibiotic exposure (p = 0.01).

Summary of statistical outcomes. Across all analyses, respiratory hypersensitivity was consistently associated with:

1. Reduced microbial richness and phylogenetic diversity;
2. Distinct gut community structures confirmed by PERMANOVA;
3. Enrichment of potentially pathogenic taxa; and

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4. Loss of protective immune modulation linked to depletion of SCFA-producing commensals.

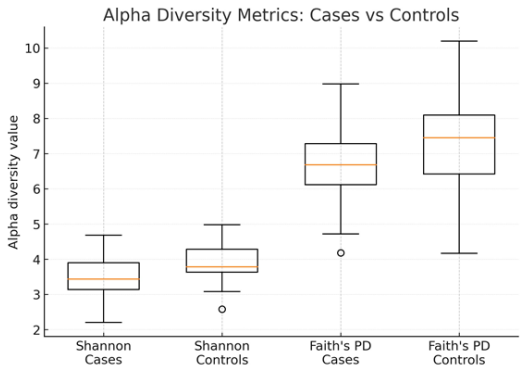

Figure 4. *Boxplots showing alpha diversity metrics (Shannon diversity index and Faith's phylogenetic diversity) in cases and controls.*

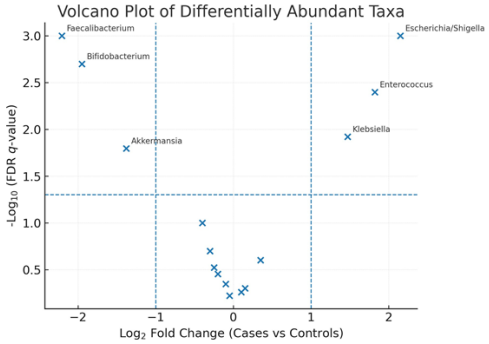

Figure 5. *Volcano plot of differentially abundant taxa between cases and controls. Taxa with statistically significant differences after false discovery rate correction (FDR q < 0.05) are highlighted.*

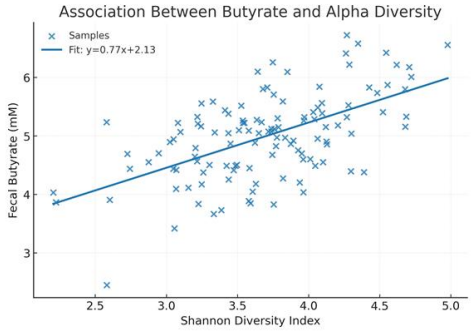

Figure 6. *Scatter plot showing the correlation between fecal butyrate concentration and Shannon diversity index.*

DISCUSSION

5.1 Key Findings and Interpretations

This case–control study provides evidence that children with respiratory hypersensitivity exhibit clear alterations in gut microbiome composition compared with age- and sex-matched controls. We observed a loss of alpha diversity, marked shifts in beta diversity, and a consistent depletion of short-chain fatty acid (SCFA)–producing taxa, including *Faecalibacterium* and *Bifidobacterium*, alongside enrichment of potentially pathogenic genera such as *Escherichia/Shigella* and *Klebsiella*. These microbial signatures were strongly associated with respiratory symptom burden, including wheezing frequency, nocturnal cough, and activity limitation. Additionally, lower fecal butyrate concentrations were linked to reduced microbial diversity and increased symptom severity, indicating a functional association between gut microbial metabolism and pediatric respiratory health.

5.2 Relevance to the Gut–Lung Axis Theory

Our findings are consistent with the emerging concept of the gut–lung axis, which proposes bidirectional immunological and metabolic communication between the gut microbiota and the respiratory system [Budden et al., 2017]. Loss of microbial diversity and depletion of butyrate-producing taxa can impair epithelial barrier integrity, reduce regulatory T-cell

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induction, and amplify systemic inflammation, contributing to heightened airway reactivity [Yu et al., 2025]. Enrichment of Enterobacteriaceae, including *Escherichia/Shigella*, may reflect dysbiosis driven by prior antibiotic exposure, aligning with evidence that early-life microbial instability increases asthma risk [Stokholm et al., 2018; Arrieta et al., 2015]. Collectively, these results support the hypothesis that gut microbial ecological shifts contribute to immune dysregulation and the pathogenesis of childhood respiratory hypersensitivity.

5.3 Clinical and Preventive Implications

Clinically, this study highlights the potential of gut microbiome composition as a biomarker for identifying children at increased risk of respiratory hypersensitivity [Shahbazi Khamas et al., 2025]. Integrating microbial signatures with clinical and environmental risk factors may enhance predictive models of disease onset and progression [Reicher et al., 2025]. Microbiome-targeted interventions—such as dietary modulation, prebiotics, or probiotics—may offer preventive strategies by increasing microbial diversity and enhancing SCFA production [Shi et al., 2024]. Furthermore, prudent antibiotic stewardship during childhood remains essential, given its strong association with dysbiosis and increased respiratory morbidity. Incorporating microbiome data into clinical guidelines for asthma and allergy management may open new avenues for precision medicine.

5.4 Limitations and Potential Confounders

Several limitations warrant consideration. First, the case–control design precludes causal inference, and reverse causality cannot be excluded [Abudari et al., 2025]. Second, stool samples represent a proxy for gut microbial dynamics and may not fully capture mucosal-associated communities. Third, although we adjusted for key confounders such as age, sex, and recent antibiotic exposure, residual confounding from diet, socioeconomic status, or unmeasured environmental factors may remain [Choi et al., 2025]. Fourth, while 16S rRNA sequencing is cost-effective, it provides limited taxonomic resolution and does not directly assess functional activity [Matchado et al., 2024]. Shotgun metagenomics and metabolomics could offer deeper insights into host–microbe pathways. Finally, although the sample size was sufficient to detect group-level differences, it may have limited power to identify more subtle associations.

5.5 Future Research Directions

Future studies should adopt longitudinal designs to clarify temporal relationships between microbiome development and respiratory outcomes. Multi-omics approaches—including metagenomics, metatranscriptomics, and metabolomics—will enable comprehensive characterization of microbial function and host–microbe interactions [Mathuria et al., 2024]. Interventional studies examining microbiome modulation strategies (e.g., high-fiber diets, synbiotics, or postbiotics) are needed to test causal effects on respiratory health. Expanding sample sizes and including diverse populations will improve generalizability and facilitate identification of population-specific microbial signatures [Joos et al., 2025; Singh et al., 2025]. Ultimately, integrating microbiome biomarkers into pediatric allergy and asthma risk stratification tools may advance translational and preventive strategies grounded in the gut–lung axis.

CONCLUSION

6.1 Summary of Major Insights

This case–control study demonstrates that respiratory hypersensitivity in children is associated with distinct alterations in gut microbiome composition. Affected children exhibited reduced alpha diversity, pronounced beta diversity shifts, enrichment of pro-inflammatory taxa such as *Escherichia/Shigella* and *Klebsiella*, and depletion of SCFA-producing commensals including *Faecalibacterium* and *Bifidobacterium*. These microbial changes correlated with symptom severity and reduced fecal butyrate levels, underscoring their functional relevance to immune regulation. Collectively, these findings highlight the critical role of microbiome ecology in shaping early-life respiratory health.

6.2 Importance of Gut Health in Childhood Allergies

These results add to growing evidence that the gut–lung axis plays a central role in pediatric allergic and respiratory diseases. Early-life disruption of the gut microbiota may bias immune development toward hypersensitivity, weaken mucosal tolerance, and increase susceptibility to airway inflammation. Maintaining gut microbial diversity through breastfeeding, dietary fiber intake, and judicious antibiotic use may therefore represent key preventive strategies against childhood allergies and asthma. Intestinal microbiome signatures may also serve as clinical biomarkers and therapeutic targets.

6.3 Final Takeaways

Overall, this study emphasizes that gut microbial diversity and composition not only reflect child health status but may also influence respiratory outcomes. While causal conclusions cannot yet be drawn, the observed associations provide a strong rationale for longitudinal and interventional research. Preservation and restoration of the gut microbiome in early childhood represent promising opportunities to reduce the burden of respiratory hypersensitivity and inform future precision-prevention strategies centered on the gut–lung axis.

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