The Role of Gut Microbiota in Early-Life Allergen Sensitization and Immune Development

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Abstract: *Background:* Early-life microbial colonisation plays a pivotal role in shaping the infant immune system and influencing susceptibility to allergic diseases.

Objective: This study aimed to establish the relationship between the characteristics of the gut microbiota and the development of allergen sensitisation in infants during their first year of life.

Methods: The research was conducted in Poland at the Medical University of Warsaw between January 2022 and May 2023. The methodology involved prospective monitoring of 112 full-term infants, with sequential collection of faecal samples and microbiota analysis using 16S rRNA gene sequencing at 1, 3, 6, and 12 months of age. Immunological assessment included the determination of total and specific IgE, flow cytometric cell typing, and multiplex analysis of serum cytokine levels.

Findings: The results indicated that infants with signs of sensitisation showed a reduction in microbial alpha-diversity from the 1st month of life (Shannon index: 2.15 vs 2.47), with statistically significant differences emerging from the 3rd month ($p<0.05$). Beta-diversity based on the Bray-Curtis metric revealed a persistent divergence in microbial communities depending on sensitisation status, most pronounced at 6 and 12 months. Sensitised infants exhibited an increased relative abundance of pro-inflammatory taxa, including *Escherichia*/*Shigella* and *Klebsiella*, whereas non-sensitised infants were dominated by immunoprotective microorganisms such as *Bifidobacterium* and *Faecalibacterium*. Furthermore, sensitised infants demonstrated early and sustained elevations in total and specific IgE, as well as a shift in the immune response towards a Th2 profile, characterised by increased levels of IL-4 and IL-5, a reduction in $CD4^+$ T cells, and an increase in the proportion of $CD19^+$ and NK cells.

Conclusion: The findings confirm the critical role of microbial diversity in immunological calibration and the development of the allergic phenotype. The results may be used in clinical practice by paediatricians, immunologists, and allergists for early diagnosis and risk assessment of allergic diseases in children, as well as in the development of microbiota-targeted interventions and sensitisation prevention strategies within primary healthcare and perinatal monitoring programmes.

Keywords: Taxonomic Shift, Pro-Inflammatory Mediators, T-Helper Imbalance, Barrier Function, Flow Cytometric Typing.

1. INTRODUCTION

The development of the immune system during early childhood represents a critically important period that determines both resistance to infections and the likelihood of developing immunopathologies, including allergic diseases. Since 2010, a steady increase in the incidence of allergic conditions among children has been observed, particularly in industrially developed countries. The early onset of allergic responses is associated with dysfunctions of the innate and adaptive immune systems, often linked to disturbances in the establishment of gut microbiota. Given that the intestine is the primary immunological barrier in early life, its microbial population plays a key role in shaping immune tolerance and maintaining the balance between inflammatory and regulatory signals.

Early, population-level interventions that stabilise infant gut ecology have clear public-health relevance, given the rising burden and long-term costs of atopic disease. Microbiota-targeted nutrition strategies - such as promoting and supporting breastfeeding, judicious maternal nutrition, and the considered use of prebiotics, probiotics, or synbiotics during defined windows of immune plasticity - offer scalable, comparatively low-risk options to enhance mucosal tolerance [1, 2]. Complementary feeding practices that diversify fibre and fermentable substrates and antimicrobial stewardship in perinatal care may further reduce dysbiosis and downstream sensitisation risk [3, 4]. Embedding these measures in routine maternal-child health pathways and coupling them with early risk stratification using simple biomarkers (e.g., diversity indices, total/specific IgE) and clinical predictors can guide targeted prevention while preserving equity.

A particularly significant concern is the reduction in microbial diversity during the 1st months of life, which is associated with impaired mechanisms of immunological tolerance [5-7]. This issue was studied by Januszkiewicz *et al.* [8], who demonstrated that infants delivered by caesarean section exhibited limited

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initial microbial colonisation and an increased risk of atopic manifestations during the first year of life. However, this study was confined to a fixed time window - the 1st month only - and did not include longitudinal tracking of microbial changes, leaving the long-term consequences of microbial deficits unresolved.

Another key issue concerns the role of opportunistic microorganisms, whose excessive presence may induce a pro-inflammatory immune profile. Dera *et al.* [9] investigated the microbiota of infants with atopic dermatitis and found an over-representation of *Klebsiella* and *Enterobacter* in their faecal samples. Despite strong correlations between inflammatory status and microbial composition, the study lacked an immunological component - no measurements of IgE or cytokine markers were conducted - which prevented the establishment of a direct link between microbiota and sensitisation. The issue of insufficient colonisation by probiotic strains, particularly *Bifidobacterium*, was the focus of the study by Cukrowska *et al.* [10]. It showed that infants with low levels of *Bifidobacterium* before 3 months of age exhibited higher concentrations of inflammatory cytokines and a predisposition towards the Th2 response. However, the retrospective design and limited dataset reduced the reliability of the findings and precluded causal inference.

Disturbances in microbial beta-diversity are also associated with immunological dysfunctions [11-13]. Borbet *et al.* [14] applied the Bray-Curtis metric to assess differences in microbiota composition between children with and without signs of allergy. They observed persistent divergences from as early as 6 months of age. However, the study did not assess alpha-diversity and failed to account for environmental factors such as feeding practices and pet exposure, which limits the interpretability of the results. Particular attention in the literature is given to the relationship between microbial colonisation and specific immune cell populations. Pirker and Vogl [15] found that a low abundance of *Faecalibacterium* was associated with a reduced $CD4^+$ T-cell population and lower IL-10 levels, promoting the development of allergic sensitisation. Nonetheless, their study did not use a comprehensive panel of immunological markers, making it impossible to assess potential compensatory mechanisms of immune regulation.

Microbiota influence on immunoglobulin production, especially IgE, is also a subject of active investigation. Zegarra-Ruiz *et al.* [16] conducted a study showing that *Escherichia*/*Shigella* dominance in the infant gut microbiota was linked to persistently elevated levels of total and specific IgE. However, the study did not consider the role of other microbial groups, particularly anaerobic symbionts, thereby limiting the understanding of complex microbial interactions. The balance between Th1 and Th2 responses in infants with different microbial profiles was addressed by Méndez *et al.* [17], who concluded that reduced microbial diversity fosters a Th2-dominant profile and elevated IL-4 and IL-5 levels. However, the study relied on a single time point without follow-up monitoring, leaving the durability of the observed changes unclear. The final aspect concerns the role of natural killer (NK) cells and B cells in the context of sensitisation. Marrs *et al.* [18] demonstrated that elevated numbers of CD19+ and NK cells correlate with microbial shifts towards pro-inflammatory taxa. However, the author did not consider the dynamics of these changes or their potential age-dependence, complicating the interpretation of their role in the development of allergic status.

Against the backdrop of fragmented existing data, it becomes evident that a comprehensive longitudinal study encompassing both microbiological and immunological monitoring of infants is a necessary step towards understanding the mechanisms of early sensitisation. This study aimed to establish the relationship between gut microbiota composition and the development of allergic sensitisation in infants during their first year of life. The research objectives included microbiological analysis of faecal samples, immunological profiling, and the correlation of findings with clinical signs of sensitisation.

2. MATERIALS AND METHODS

The study was conducted between January 2022 and May 2023 at the Medical University of Warsaw, Poland, with the participation of the Departments of Microbiology, Immunology, and Paediatrics. The study protocol was approved by the Bioethics Committee of the Medical University of Warsaw (No. KB/87/2021), and all procedures complied with the provisions of the Declaration of Helsinki (2013 version) [19]. Written informed consent was obtained from all newborns' parents before data collection. The study included 112 full-term neonates (56 boys and 56 girls) born without complications or pathologies, with a birth weight between 2800 and 4000 g. Inclusion criteria were gestational age of at least 37 weeks, no maternal antibiotic use in the 30 days before delivery, and

absence of infections in the newborn during the first two weeks of life. Exclusion criteria included congenital anomalies, chronic illnesses, immunodeficiencies, admission to intensive care, and parental refusal to participate. Participants were monitored from birth to 12 months of age, with periodic collection of clinical, microbiological, and immunological data.

Faecal samples were collected at 1, 3, 6, and 12 months in sterile Sarstedt containers (Germany) and immediately frozen at -80°C. DNA was extracted using the QIAamp DNA Stool Mini Kit (QIAGEN, Germany) via a sorbent-column purification method. Amplification of the 16S rRNA gene (V3-V4 regions) was performed using the Illumina MiSeq platform (Illumina, USA), and sequence analysis was conducted using QIIME 2 software, version 2022.2. Taxonomic classification was based on the Greengenes database. Quantitative characteristics of the microbiota (alpha- and beta-diversity, relative taxon abundance) were calculated following read count normalisation. Microbiota stability was assessed using Shannon and Simpson indices [20], and group differences were evaluated using the Bray-Curtis metric [21].

Immunological assessment included measurement of total IgE and specific IgE (to milk proteins, egg, house dust mite, and tree pollen) using chemiluminescent immunoassay on the Immulite 2000 automated analyser (Siemens Healthcare Diagnostics, USA). Immunophenotyping of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD16^+/56^+$ cells was conducted by flow cytometry using the BD FACSCanto II analyzer (BD Biosciences, USA) and the BD Multitest reagent kit. Additionally, serum levels of pro-inflammatory cytokines IL-4, IL-5, IL-10, IFN-γ, and TNF-α were measured using multiplex analysis on the Luminex 200 platform (Luminex Corporation, USA).

To address confounding, we prespecified an adjustment set comprising feeding type (exclusive breastfeeding, mixed, or formula), cumulative breastfeeding duration (weeks), household exposures (pet ownership, ≥1 smoker in household, and self-reported antiseptic use frequency), living conditions (apartment/house; number of cohabitants), and postnatal antibiotic exposure in the infant. Environmental variables were captured at 3-month intervals and modelled as time-varying covariates; feeding type was encoded as a categorical factor, breastfeeding duration as a continuous measure, pet/smoking as binary indicators, and antiseptic use as an ordinal frequency score. The primary endpoint - sensitisation at 12 months (specific IgE≥0.35 kU/L to ≥1 allergen) - was analysed with multivariable logistic regression including all prespecified confounders irrespective of univariable significance. In secondary longitudinal models of sensitisation status across 3, 6, and 12 months, we fitted mixed-effects logistic regressions with a random intercept for child and a fixed effect for visit to account for within-infant correlation and secular time. Microbiota predictors (alpha-diversity indices; taxa relative abundances) were standardised (z-scores) and, for compositional features, centred log-ratio transformed; all models used robust standard errors clustered by infant. Multicol-linearity was assessed via variance inflation factors (VIF < 5 for all terms). Missing covariate data (<10% per variable) were handled by multiple imputation by chained equations (m=20) under missing-at-random assumptions, with sensitivity analyses using complete cases and inverse probability weighting for attrition. An additional sensitivity analysis lagged time-varying covariates by one visit (e.g., antibiotic exposure between months 0 and 3 predicting outcomes at month 6) to mitigate reverse causation. Statistical significance was evaluated at two-sided α=0.05.

3. RESULTS

From the 1st month of life to the end of the observation period, children with allergic sensitisation had significantly different gut microbiota alpha- and beta-diversity. Sensitised newborns had decreased Shannon and Simpson indices from month one, indicating diminished species richness and taxonomic dominance. Bray-Curtis beta-diversity demonstrated continuous grouping of samples by sensitisation state, becoming more prominent at 6 and 12 months. Early statistical significance was observed in month 3 ($p<0.05$). Reduced microbial diversity in early infancy may predict allergic reactions (Table **1**).

The first-year alpha- and beta-diversity indices of newborn gut microbiota showed significant differences between children with verified allergy sensitisation and their non-sensitised peers. Sensitised infants had lower alpha-diversity in the 1st month: Shannon index = 2.15 and Simpson index = 0.62, compared to 2.47 and 0.68 in the non-sensitised group. These numbers suggest lesser species richness and a narrow microbiome, which may be important during immune system development. Beta-diversity differences (Bray-Curtis) have not yet reached statistical significance ($p=0.071$), but sensitised infants were already forming a separate microbial cluster.

Table 1: Alpha- and Beta-Diversity Indices of Gut Microbiota in Sensitised and Non-Sensitised Children

Age (months)	Group	Shannon Index (M±SD)	Simpson Index (M±SD)	Bray-Curtis (p-value)
1	Sensitised	2.15±0.34	0.62±0.09	0.071
	Non-sensitised	2.47±0.29	0.68±0.08	-
3	Sensitised	2.36±0.31	0.65±0.07	0.032
	Non-sensitised	2.71±0.26	0.72±0.06	-
6	Sensitised	2.44±0.28	0.66±0.08	0.017
	Non-sensitised	2.89±0.27	0.74±0.05	-
12	Sensitised	2.53±0.3	0.67±0.06	0.009
	Non-sensitised	3.02±0.25	0.77±0.04	-

Note: p<0.05 - statistically significant.
Source: compiled by the author.

By the 3rd month, sensitised children had decreased alpha-diversity (Shannon: 2.36; Simpson: 0.65), while the non-sensitised group had sustained microbial diversification (Shannon: 2.71; Simpson: 0.72). First, beta-diversity analysis revealed a statistically significant difference (Bray-Curtis p=0.032), indicating divergence in microbial community composition. This may be a crucial time for immunological memory and allergy resistance. At this point, reduced taxonomic diversity may decrease immunological tolerance and enhance allergic Th2-helper responses.

At 6 months, the Shannon index became even more distinct: 2.89 in the non-sensitised group and 2.44 in the sensitised group. Similar dynamics were seen for the Simpson index (0.74 vs. 0.66). Bray-Curtis discrepancies became more significant (p=0.017), indicating a more persistent and structured microbiota composition divergence. The introduction of supplemental nutrition at this period may further change immune reactivity, making our findings significant.

Despite increasing microbial diversity in both groups by 12 months, sensitised children had significantly lower values (Shannon: 2.53; Simpson: 0.67) than the control group (Shannon: 3.02; Simpson: 0.77). Bray-Curtis discrepancies peaked (p=0.009), showing sensitised newborns had a unique microbial makeup. These data support the idea that early microbial diversity loss represents a chronic dysbiotic pattern that may impede immune induction and tolerance.

Thus, the results suggest that microbial diversity in early infancy serves not only trophic and metabolic functions but also plays a critically important role in immunological programming. Persistently reduced alpha-diversity and stable divergence in beta-diversity among sensitised infants may be considered predictors of increased risk for developing allergic diseases.

At all observation points, children with allergic sensitisation had a significantly different gut microbiota taxonomic makeup. At 1-3 months, sensitised infants had a prevalence of *Clostridium sensu stricto*, Escherichia/Shigella, and Enterococcus, which cause inflammation and impair immunological tolerance. Immunomodulatory Bifidobacterium and Bacteroides dominated non-sensitised neonates. At 6 and 12 months, sensitised children had a high relative abundance of Proteobacteria, particularly Klebsiella and Citrobacter, while non-sensitised children gradually increased *Lachnospiraceae* and *Ruminococcaceae*, promoting a balanced immune profile (Appendix **A**).

A comprehensive investigation of gut microbiota taxonomic makeup showed consistent differences between sensitised and non-sensitised children from 1 to 12 months of age. Differences affected dominating genera and less abundant yet functionally significant taxa. Sensitised newborns had significantly larger amounts of *Escherichia/Shigella* (14.2% vs. 5.8%; p=0.004) and *Enterococcus* (9.5% vs. 3.6%; p=0.011) in the 1st month of life than the control group. Known to damage intestinal epithelial integrity, these bacteria increase pro-inflammatory mediators and TLR4 receptor expression, potentially increasing immune system susceptibility to external antigens. In contrast, *Bifidobacterium* (21.7% vs. 12.4%; p=0.008) and *Bacteroides* (14.5% vs. 6.8%; p=0.002), which promote immunological tolerance and decrease Th2 responses, dominated non-sensitised newborns.

By 3 months, sensitised infants had more inflammatory taxa in their microbial profile. *Clostridium*

sensu stricto (11.1% vs. 4.2%; p=0.006) and *Klebsiella* (13.9% vs. 6.7%; p=0.009), which produce lipopolysaccharides, activate immature dendritic cells, and induce IgE production, increased. *Veillonella*, a metabolically active species that sustains microbial balance without initiating inflammatory cascades, increased in non-sensitised infants (9.6% vs. 4.5%; p=0.015).

By 6 months, differences became more pronounced. Sensitised infants were dominated by *Citrobacter* (10.8% vs. 3.9%; p=0.001), *Klebsiella* (15.7% vs. 5.2%; p=0.003), and *Streptococcus* (7.3% vs. 3.1%; p=0.018) - genera associated with dysbiosis and disrupted immune regulation. Conversely, in the control group, the relative abundance of *Lachnospira* (8.9% vs. 3.2%; p=0.004), *Faecalibacterium* (7.8% vs. 2.7%; p=0.005), and *Bacteroides* increased, indicating favourable microbiota maturation. Of particular importance is the presence of *Faecalibacterium prausnitzii*, a key anti-inflammatory symbiont.

At 12 months, sensitised infants continued to show dominance of Proteobacteria - *Klebsiella* (16.3% vs. 4.6%; p=0.002), *Proteus* (5.9% vs. 2.1%; p=0.014), and *Enterobacter* (9.1% vs. 3.4%; p=0.007) - reflecting a persistent pro-inflammatory microbial pattern. Meanwhile, the control group was dominated by *Ruminococcus* (10.7% vs. 4.3%; p=0.003), *Roseburia* (9.3% vs. 3.8%; p=0.006), and *Akkermansia* (6.9% vs. 2.4%; p=0.011). The latter species, *Akkermansia muciniphila*, reinforces the mucosal barrier and supports regulation of metabolic and inflammatory processes, confirming its role in maintaining immune tolerance.

Thus, the observed differences suggest that specific taxa within the gut microbiota may not only serve as markers of immune status but also actively participate in modulating early stages of immune system development. The predominance of Proteobacteria and the reduction in anti-inflammatory anaerobes in sensitised children reflect impaired microbial maturation, potentially predisposing them to allergic diseases.

Total and specific IgE levels differed significantly between allergic sensitised and non-sensitised babies. The investigation did not include IgE levels in the 1st month of life, which fall within the physiological range for neonates and are primarily due to transplacental transfer of maternal immunoglobulins. To scientifically test sensitisation and eradicate passive immunity, the study began at 3 months of age, when endogenous IgE production begins. Sensitised infants' total IgE concentrations increased steadily from three to 12 months. Most sensitised children produced cow's milk protein and egg-specific IgE by 6 months. The non-sensitised group had normal specific antibody levels, but sensitised infants exceeded 0.35 kU/L. Food allergies, house dust mites, and tree pollen were the most common triggers, especially later in follow-up. Some newborns develop a hyperreactive humoral immune response and polyvalent sensitisation (Table **2**).

The synchrony between rising IgE concentrations and the taxonomic shift towards pro-inflammatory bacterial genera underscores a plausible causal link between microbial imbalance and allergic sensitisation. Infants with a dominance of *Escherichia/Shigella*, Klebsiella, and *Citrobacter* exhibited the steepest increases in total and specific IgE, suggesting that these taxa may act as triggers of Th2-mediated humoral activation. In contrast, children with stable colonisation by *Bifidobacterium*, *Faecalibacterium*, and *Akkermansia* maintained lower IgE values throughout follow-up, indicating that microbial communities with anti-inflammatory potential support immune tolerance. This parallel trajectory of microbial dysbiosis and

Table 2: Levels of Total and Specific IgE in Children with and without Sensitisation (Mean Values±SD)

Age (months)	Group	Total IgE (kU/L)	IgE to milk	IgE to egg	IgE to dust mite	IgE to pollen
3	Sensitised	12.7±3.5	0.42±0.18	0.37±0.14	0.29±0.1	0.21±0.08
	Non-sensitised	5.2±1.8	0.18±0.09	0.15±0.07	0.11±0.06	0.09±0.04
6	Sensitised	25.4±4.8	0.65±0.22	0.58±0.19	0.47±0.16	0.39±0.12
	Non-sensitised	9.3±2.7	0.24±0.11	0.21±0.08	0.19±0.07	0.14±0.06
12	Sensitised	38.9±6.2	0.84±0.27	0.73±0.23	0.61±0.21	0.52±0.18
	Non-sensitised	13.8±3.4	0.31±0.13	0.28±0.1	0.23±0.09	0.19±0.07

Source: compiled by the author.

immunoglobulin escalation provides mechanistic evidence that early disruptions in gut ecology can initiate the pathogenic cascade leading to allergy development.

The immunological study of total and specific IgE levels showed significant differences between infants with allergic sensitisation and those without hypersensitivity, starting early postnatally and increasing with age. At 3 months, sensitised children had a mean total IgE level of 12.7 kU/L, more than twice that of the non-sensitised group (5.2 kU/L), showing early humoral immune system activation via IgE. In certain patients, elevated levels of specific IgE to cow's milk proteins (0.42 kU/L) and egg (0.37 kU/L) indicated food sensitisation before supplemental feeding. While most newborns' IgE levels to house dust mite and tree pollen stayed below the threshold, they did increase.

By 6 months, intergroup differences increased. Sensitised infants had 25.4 kU/L total IgE, while non-sensitised counterparts had 9.3 kU/L. Milk- and egg-protein-specific IgE levels increased significantly (0.65 and 0.58 kU/L), suggesting progressive sensitisation to key dietary allergens. IgE to dust mite (0.47 kU/L) and pollen (0.39 kU/L) increased, suggesting cross-reactivity or early polyvalent allergic sensitivity. Non-sensitised individuals had specific IgE levels below the clinical sensitisation threshold of 0.35 kU/L, indicating no allergic responses.

At 12 months, group differences peaked. Sensitive children had kU/L total IgE, almost three times greater than the control group (13.8). All tested allergens had elevated specific IgE levels: milk (0.84 kU/L), egg (0.73), dust mite (0.61), and pollen (0.52). This profile suggests polyvalent sensitisation, typical of atopic youngsters. Sensitisation to inhalant allergens at such an early age may be due to both environmental exposure and a genetic propensity to a Th2-dominant immune response, defined by elevated IL-4 production and B cell class switching to IgE.

Comparing the three time points demonstrates that sensitised newborns activate the IgE-mediated immune response to food and inhalant allergens early and progressively. The non-sensitised group's IgE levels grew with age but stayed within physiological limits, demonstrating immunological development without severe sensitisation. These data suggest that higher total and specific IgE levels in early infancy may indicate allergy propensity and a nascent atopic phenotype.

Sensitised children have different immune response traits, as shown by lymphocyte immunophenotyping and serum cytokine profiling. Since the immune system is reconstructing after birth, these analyses were not done at 1 month. Early measurements may not reflect stable immunological patterns and complicate sensitisation interpretation. Thus, assessments began at 3 months, when immunological profiles are more useful for intergroup comparisons. By 3 months, sensitised infants had fewer CD4+ T cells and more CD19+ B cells and NK cells (CD16+/56+). These alterations were accompanied by increased IL-4 and IL-5 levels, which mediate the Th2 response. IFN-γ and TNF-α levels decreased compared to the non-sensitized group, whereas IL-10 levels fluctuated. These discrepancies increased after 6 and 12 months, indicating Th2-dominant immune activation and pro-inflammatory/regulatory signal imbalance (Table **3**).

In the first year of life, sensitised and non-sensitised newborns had significantly different immune profiles,

Table 3: Immune Profile in Children with and without Sensitisation (Mean Values±SD)

Age (months)	Group	CD3+ (%)	CD4+ (%)	CD8+ (%)	CD19+ (%)	CD16+/56+ (%)	IL-4 (pg/mL)	IL-5	IL-10	IFN-γ	TNF-α
3	Sensitised	62.3±5.4	30.1±3.2	24.6±2.8	16.7±1.9	11.3±1.6	6.5±1.1	4.2	3.1	2.7	3.8
	Non-sensitised	66.8±4.7	35.4±2.9	23.9±2.5	13.5±1.6	8.2±1.4	3.2±0.9	2.1	3.6	4.1	5.3
6	Sensitised	64.1±5	29.3±3.5	25.7±3.1	17.8±2	12.4±1.8	7.4±1.3	4.8	2.9	2.4	3.2
	Non-sensitised	67.9±4.3	34.6±3	24.2±2.6	13.1±1.7	8.6±1.3	3.5±1	2.4	3.8	4.3	5.6
12	Sensitised	63.4±5.2	28.7±3.8	26.1±3.2	18.4±2.2	13.2±1.9	7.9±1.4	5.1	2.8	2.3	3
	Non-sensitised	68.2±4.5	33.8±3.2	24.5±2.7	12.8±1.6	8.3±1.2	3.6±0.9	2.5	3.9	4.5	5.7

Source: compiled by the author.

showing an imbalance in innate and adaptive immunity. At 3 months, sensitised newborns had 30.1% fewer CD4+ T helper cells, indicating decreased immunological coordination. High CD19+ B cells (16.7%) and CD16+/56+ NK cells (11.3%) indicated humoral and innate immunological activation. The CD8+ cytotoxic T cell proportion was somewhat greater than in the non-sensitised group (24.6% vs 23.9%), but did not alter dynamically.

The cytokine profile validated a Th2-dominant immune response. High amounts of IL-4 (6.5 pg/mL) and IL-5 (4.2 pg/mL) activated IgE production, eosinophilic infiltration, and mucosal hyperreactivity. These cytokines are essential mediators in allergy pathophysiology and immunoglobulin isotype shift to IgE. Compared to the non-sensitised group, IFN-γ (2.7 pg/mL) and TNF-α (3.8 pg/mL), which are linked to Th1 responses and antiviral defence, were considerably reduced (4.1 and 5.3 pg/mL). The universal regulatory cytokine IL-10 was also reduced (3.1 versus 3.6 pg/mL), suggesting poor immune response modulation.

By 6 months, discrepancies increased. In sensitised youngsters, CD4+ levels declined to 29.3%, while CD19+ B cells rose 17.8%, indicating a dominating B cell response. In the absence of regulatory control, NK cell numbers rose to 12.4%, probably due to compensatory activation of innate effectors. Both IL-4 and IL-5 increased to 7.4 and 4.8 pg/mL, indicating Th2 activation. In contrast, IFN-γ and TNF-α decreased to 2.4 and 3.2 pg/mL. Chronic inflammation and impaired immunological tolerance may result from IL-10's decline (2.9 pg/mL).

Sensitised children's immunological profiles remained dysregulated at 12 months. The humoral component remained dominant as CD4+ T cells declined to 28.7% and CD19+ B cells reached 18.4%. The maximal CD16+/56+ NK cell rate was 13.2%, indicating increased innate effector activation with prolonged antigenic stimulation. IL-4 and IL-5 levels rose to 7.9 and 5.1 pg/mL, but IFN-γ and TNF-α remained low (2.3 and 3 pg/mL). Regulatory anti-inflammatory responses were impaired, as IL-10 levels remained below those of the non-sensitised group.

From the 1st month of life, allergically sensitised newborns have a consistent coupling of an immature, dysbiotic microbiota and a Th2-skewed immune phenotype that strengthens through 6 and 12 months. Sensitised newborns had lower alpha-diversity (Shannon, Simpson) and early beta-divergence (Bray-Curtis, significant by 3 months), suggesting a predetermined dysbiotic trajectory. Taxonomically, pro-inflammatory and opportunistic taxa (*Escherichia/Shigella*, *Enterococcus*, later *Klebsiella*, *Citrobacter, and Proteus*) were enriched. At the same time, immunoregulatory anaerobes (*Bifidobacterium*, *Bacteroides*, members of *Lachnospiraceae* and *Ruminococcaceae*, *Faecalibacterium, and Akkermansia*) declined, indicating loss of colonisation resistance and tolerance signalling. Simultaneously, we observed a gradual increase in total and specific IgE (milk, egg, mite, pollen) and a Th2-dominant immune profile, including lower CD4+ proportions, higher CD19+ B and NK cells, increased IL-4/IL-5, and decreased IFN-γ/TNF-α and IL-10. Microbiome and immunological modifications are synchronised, so lower alpha diversity and specific compositional signatures may indicate sensitisation risk, whereas IgE and cytokine profiles indicate an activated pathogenic pathway. These findings suggest early risk stratification using diversity indices, key taxa trajectories, and simple immune biomarkers, as well as plausible interventions within first-year “critical windows” like butyrogenic community-supporting diets, cautious perinatal antimicrobial stewardship, and pre-/probiotic use.

4. DISCUSSION

The presented findings indicate a stable association between reduced intestinal microbiota diversity and the occurrence of allergic sensitisation in infants during the first year of life. Already in the 1st month of life, a notable decrease in alpha-diversity indices was observed in sensitised children, supporting the hypothesis that early microbial deficiency may act as a contributing risk factor. These observations are in line with Vercelli [22], who reported similar trends of reduced microbial diversity among infants predisposed to allergic conditions. The study by Oliva *et al.* [23] further highlighted the potential influence of dominant taxa on susceptibility to allergic pathology, which aligns with the higher relative abundance of Escherichia/ Shigella observed in sensitised children in the present dataset. These findings underscore the role of gut microbiota composition as a potential determinant in shaping early immunological development.

However, as demonstrated by Davis *et al.* [24], greater microbial diversity does not uniformly confer protection, particularly under conditions of postnatal colonisation stress. Nonetheless, the results of the present study indicate consistent divergence in microbial profiles between sensitised and non-

sensitised infants by the 3rd month of life, with statistically significant differences in beta-diversity. These findings suggest that the variations observed reflect functional imbalances within microbial communities rather than transient ecological fluctuations. The consistency of these trends with the results of Reynolds and Bettini [25], who examined the relationship between dysbiotic clustering and Th2 cell differentiation, lends further plausibility to the functional interpretation of these microbial patterns.

By 6 months of age, these differences became more pronounced, particularly in relation to complementary feeding practices. The predominance of *Klebsiella* and *Citrobacter* in sensitised children may reflect a loss of colonisation resistance, a phenomenon consistent with the transcriptomic findings of Ignacio *et al.* [26], who reported that similar microbial profiles were accompanied by reduced expression of genes encoding antimicrobial peptides and tolerance-associated molecules. In contrast, the relative increase of *Lachnospiraceae* and *Ruminococcaceae* in the non-sensitised group - families known for their contribution to butyrate synthesis - suggests the preservation of microbial functions linked to immune regulation, as also discussed by Campbell *et al.* [27]. While Saeed *et al.* [28] questioned whether reduced microbial diversity directly mediates sensitisation risk, the present findings, which reveal consistent and temporally stable differences in both microbial composition and cytokine activity, strengthen the argument for a meaningful association between microbial features and immune outcomes.

Taxonomic analysis further identified bacterial genera potentially influencing immune system trajectories. The predominance of *Escherichia/Shigella*, *Enterococcus*, and *Clostridium sensu stricto* in sensitised infants parallels the observations of Chen *et al.* [29], who described their role in activating pro-inflammatory signalling pathways. Huang *et al.* [30] noted that *Klebsiella* species can modulate dendritic cell activity and promote a Th2-skewed cytokine milieu. Conversely, the relative abundance of *Bifidobacterium* and *Bacteroides* in non-sensitised infants aligns with prior evidence from Alcazar *et al.* [31] and Knoop *et al.* [32], demonstrating that these taxa contribute to short-chain fatty acid production and support Treg differentiation, both processes that foster immune tolerance. The dominance of *Bifidobacterium* in non-sensitised infants from the 1st month of life in this study thus coincides with a more balanced immunological state.

The subsequent rise of *Lachnospira*, *Faecalibacterium*, and *Ruminococcus* at 6-12 months in non-sensitised children corresponds to the findings of Huang *et al.* [33], who emphasised their role in establishing metabolic stability and mitigating inflammatory signalling. Particularly, *Faecalibacterium* is known to suppress pro-inflammatory cytokine gene expression, which could partly explain its association with more favourable immune outcomes. Therefore, although the present data cannot confirm direct causation, the sustained presence of these genera in non-sensitised infants may represent a microbiological context conducive to balanced immune maturation. By contrast, Xie and Liu [34] did not observe a consistent relationship between *Faecalibacterium* levels and allergy risk, possibly due to their cross-sectional design. The longitudinal framework of the current study provides a more nuanced perspective on these dynamic processes.

Immunological analyses indicated an early predominance of Th2-type responses in sensitised infants. Elevated IL-4 and IL-5 levels at 3 months suggest increased humoral activation and reduced regulatory control, echoing the findings of Mousavian *et al.* [35]. Similarly, Magalhães *et al.* [36] linked lower IFN-γ and TNF-α concentrations with heightened allergy risk, a pattern mirrored here. According to Buchholz *et al.* [37], such cytokine imbalances may reflect reduced exposure to immunomodulatory microbial signals - an interpretation supported by the concurrent microbiota data in this cohort.

Immunophenotyping further revealed a consistent reduction in $CD4^+$ T cells and elevated $CD19^+$ B cells among sensitised infants, resembling the immunological profile described by Xu *et al.* [38] in atopic dermatitis. Increased NK cell counts, as observed here, may represent a compensatory response to insufficient Treg activity, as proposed by Kartjito *et al.* [39]. Nonetheless, Moriki *et al.* [40] noted that similar increases may precede allergic manifestations, highlighting the complexity of early immune regulation. Collectively, these patterns suggest disturbances in the coordination between innate and adaptive immunity rather than a singular mechanistic pathway.

An alternative perspective is offered by Wang *et al.* [41], who proposed that early Th2 predominance may reflect a physiological adaptation aimed at preventing excessive inflammatory damage in neonatal tissues. While such a transient response may be adaptive, the

persistence and amplification of Th2 bias observed here, alongside rising IgE levels and declining regulatory cytokines, indicate that the balance may shift towards maladaptive immune activation in certain infants. Notably, the data support a sustained association - rather than a proven causal sequence - between microbial composition, immune regulation, and allergic sensitisation risk.

Elevated allergen-specific IgE levels further reinforce the observed linkage between immune hyperreactivity and microbial patterns. This aligns with findings from Tun *et al.* [42] and Yang and Cong [43], who both reported that early IgE sensitisation correlates with increased risk for subsequent allergic rhinitis and asthma. In the present study, such associations highlight a potentially predictive, albeit non-deterministic, relationship between early microbial-immune profiles and later atopic outcomes.

Overall, the data demonstrate interrelated patterns between reduced microbial diversity, enrichment of pro-inflammatory taxa, activation of humoral immune pathways, and Th2-skewed cytokine profiles. While causality cannot be inferred, these associations collectively suggest that early-life microbiota composition and immune development evolve in a coordinated manner. This reinforces the relevance of the gut microbiota as a potential modulator of immune maturation and points toward the need for future interventional research to determine whether modifying early microbial environments may contribute to more balanced immune trajectories and potentially lower sensitisation risk.

5. CONCLUSIONS

The results of the study indicate that the development of intestinal microbiota in early infancy plays an essential role in shaping immune trajectories and the likelihood of allergic sensitisation. In children with signs of sensitisation from the first month of life, a persistent decrease in alpha-diversity indices was observed: the Shannon index was 2.15 ± 0.34 and the Simpson index 0.62 ± 0.09, whereas in non-sensitised children these values were higher - 2.47 ± 0.29 and 0.68 ± 0.08, respectively. This pattern, suggesting reduced microbial richness and dominance of a limited set of taxa, persisted and became more pronounced by one year of age (Shannon: 2.53 ± 0.30 vs. 3.02 ± 0.25; Simpson: 0.67 ± 0.06 vs. 0.77 ± 0.04). Beta-diversity analysis using the Bray-Curtis metric demonstrated a progressive divergence of microbial communities between groups: while at one month the differences were not statistically significant ($p = 0.071$), they became significant at three months ($p = 0.032$), increased by six months ($p = 0.017$), and reached a maximum by twelve months ($p = 0.009$), reflecting early and stable differentiation of microbial ecosystems in sensitised infants.

The taxonomic composition of the microbiota also differed markedly. Sensitised children exhibited enrichment in pro-inflammatory genera such as *Escherichia/Shigella*, *Klebsiella*, *Enterococcus*, and *Clostridium sensu stricto*, while taxa with immunomodulatory potential, including *Bifidobacterium*, *Faecalibacterium*, *Lachnospira*, *Roseburia*, and *Akkermansia muciniphila*, dominated non-sensitised peers. These microbial distinctions were paralleled by elevated total and specific IgE levels in sensitised children from as early as three months (12.7 ± 3.5 kU/L vs. 5.2 ± 1.8 kU/L), rising by twelve months (38.9 ± 6.2 kU/L vs. 13.8 ± 3.4 kU/L), and by a cytokine pattern indicative of Th2-skewed immunity.

While the findings demonstrate robust and temporally consistent associations between microbial diversity, immune parameters, and sensitisation outcomes, these should not be interpreted as evidence of direct causation. The absence of genetic, dietary, and environmental stratification limits inferences about the directionality of the observed relationships. Nonetheless, the concurrent trajectories of microbial imbalance, IgE elevation, and Th2-biased immunity suggest biologically relevant interactions that merit further exploration.

From a clinical perspective, these insights underscore the potential utility of early microbial and immunological profiling for identifying infants at higher risk of allergic sensitisation. Routine monitoring of microbiota diversity indices and early immune markers - such as total IgE, specific IgE to common food allergens, and Th2-associated cytokines - could contribute to more precise risk stratification in paediatric settings. Preventive nutritional strategies, including sustained breastfeeding support, timely diversification of complementary feeding, and consideration of probiotic or prebiotic supplementation during critical windows of immune development, may help maintain microbial equilibrium and promote mucosal tolerance. Future longitudinal and interventional studies will be essential to assess the feasibility, safety, and efficacy of such measures and to translate these associations into evidence-based recommendations for paediatric allergy prevention.

AUTHOR'S CONTRIBUTIONS

MZL conceptualized the research, designed the study, conducted the primary data analysis and interpretation, contributed to the design, data collection, drafting of the manuscript, and its final editing.

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None.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ETHICS

The study protocol was approved by the Bioethics Committee of the Medical University of Warsaw (No. KB/87/2021), and all procedures complied with the provisions of the Declaration of Helsinki (2013 version).

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this study are available in the article.

Appendix A: Key Differences in the Taxonomic Composition of Gut Microbiota in Sensitised and Non-Sensitised Children (Mean Relative Abundance, % ±SD; p-Values)

Age (months)	Taxon	Sensitised (%)	Not sensitised (%)	p-value
1	*Escherichia/Shigella*	14.2±3.1	5.8±2	0.004
	Enterococcus	9.5±2.7	3.6±1.5	0.011
	Bifidobacterium	12.4±3.9	21.7±4.5	0.008
	Bacteroides	6.8±2.2	14.5±3.7	0.002
3	*Clostridium sensu stricto*	11.1±2.8	4.2±1.6	0.006
	Klebsiella	13.9±3.4	6.7±2.1	0.009
	Veillonella	4.5±1.3	9.6±2.5	0.015
6	*Citrobacter*	10.8±2.6	3.9±1.4	0.001
	Klebsiella	15.7±4.1	5.2±2	0.003
	Streptococcus	7.3±1.8	3.1±1.2	0.018
	Lachnospira	3.2±1	8.9±2.4	0.004
	Faecalibacterium	2.7±0.9	7.8±2.1	0.005
12	*Klebsiella*	16.3±3.9	4.6±1.8	0.002
	Proteus	5.9±1.5	2.1±0.9	0.014
	Enterobacter	9.1±2.3	3.4±1.2	0.007
	Ruminococcus	4.3±1.2	10.7±3.1	0.003
	Roseburia	3.8±1.1	9.3±2.6	0.006
	Akkermansia	2.4±0.8	6.9±2.3	0.011

Note: taxa with statistically significant differences between groups ($p<0.05$), identified based on relative abundance analysis using QIIME 2, Bray-Curtis metric, and validated by PERMANOVA.
Source: compiled by the author.

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