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# Association of asthma and anti-asthmatic therapy with oral health and microbiota alterations in children - a cross-sectional study

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## Abstract

**Background** Asthma and its treatments are recognized as risk factors for oral health in children. The composition of oral microbiota is linked to asthma, as well as to the development of caries and other oral conditions. This study aimed to assess the impact of asthma and anti-asthmatic therapy on children`s oral health and explore the role of salivary microbiota in this relationship.

**Methods** A cross-sectional study was conducted, including 100 children with asthma, aged 6 to 18 years, and 50 matched healthy controls. Oral health was assessed using the decayed, missing and filled teeth (DMFT/dft) index, the simplified oral hygiene index (OHI-S) to evaluate dental plaque and calculus, and the gingival index. Saliva flow rate and pH were measured, and the salivary microbiota composition was analyzed using 16 S amplicon sequencing.

**Results** Children with asthma had a median DMFT/dft index of 4, versus 0 in controls ($p < 0.01$), a median OHI-S of 0.9 versus 0 ($p < 0.01$) and a median gingival index of 1 versus 0 ($p < 0.01$). Daily use of inhaled corticosteroids (ICS) was associated with a higher median OHI-S (1 vs. 0.8, $p < 0.01$) but had no effect on the DMFT/dft or gingival index. The median unstimulated saliva flow rate and pH were 1.3 mL and 6.25 in children with asthma compared to 2.5 mL and 7.00 in controls ($p < 0.01$ for both). Children with asthma daily using ICS had a lower saliva flow (1.0 mL) and pH (6.25) compared to non-ICS users (1.5 ml and 6.75, $p < 0.01$ for both). The salivary microbiota composition in children with asthma differed significantly from controls ($p < 0.01$), showing increased abundances of *Streptococcus*, *Gemella*, and *Streptobacillus*. Alpha diversity was lower in children with asthma, with reduced richness ($p < 0.01$) and evenness ($p = 0.012$). However, no significant associations were identified between salivary microbiota and specific oral health indicators.

**Conclusions** Asthma negatively affects children`s oral health, including reduced salivary flow, and is associated with alterations in the oral microbiota. However, the development of caries in children with asthma cannot be attributed solely to medications or microbiota alterations. Therefore, meticulous oral hygiene and other preventive measures are especially important in children with asthma.

**Keywords** Asthma, Anti-asthmatic agents, Child, Microbiota, Oral health, Saliva

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## Introduction

Asthma is one of the most prevalent chronic diseases in childhood, affecting up to 10% of children aged five years and older. It is a chronic inflammatory condition of the bronchial tract with an unclear aetiology, resulting from a complex interplay of genetic and environmental factors in early life, that remain incompletely understood, despite extensive research. Key contributors include viral respiratory infections, air pollution and reduced microbial exposure, as proposed by the "hygiene hypothesis" [1–3].

Oral microbial dysbiosis, an ecological imbalance in the composition and function of the oral microbiota, is now recognized as a central mechanism in the development of dental caries, one of the most widespread chronic conditions. This shift toward pro-cariogenic species is influenced by factors such as diet, oral hygiene, salivary flow, and environmental exposures. Among the key bacterial contributors is *Streptococcus mutans*, which metabolizes dietary sugars into acids, leading to the demineralization of dental hard tissues [4, 5]. Genetic predisposition has also been implicated in caries susceptibility [6].

Both asthma and its treatments can adversely affect oral health. Studies consistently report higher dental plaque and calculus indices in children with asthma compared to healthy peers. Children with asthma often exhibit lower salivary pH and elevated levels of cariogenic bacteria, such as *S. mutans* and *Lactobacillus*, further promoting dysbiotic state [7, 8].

Immunosuppressive therapies, including corticosteroids commonly used in asthma management, can also exacerbate gingivitis and periodontal disease [9–11]. Inhaled corticosteroids (ICS), the cornerstone of asthma therapy, predispose individuals to conditions like oral candidiasis [12]. Additionally, short-acting beta-2 agonists (SABA), used for acute asthma exacerbations, and long-acting beta-2 agonists (LABA), administered in combination with ICS for maintenance therapy, may also negatively impact oral health [3, 13].

Recent evidence indicates associations between the human microbiota and asthma pathogenesis; however, the specific mechanisms underlying these associations remain elusive. The gut microbiota plays a central role in the "hygiene hypothesis," modulating the balance between allergen tolerance and sensitization, which can influence the development of allergic airway diseases [14, 15]. The oropharyngeal microbiota has shown cluster-based predictive value for asthma development in children [16]. Similarly, the oral microbiota, due to its direct connection to the respiratory system, may significantly influence respiratory health [17]. Recent studies show robust associations between inflammation and allergic conditions and altered oral microbiota composition and diversity [18–20]. However, since asthma is closely linked with poorer oral health, it can be challenging to distinguish between disease-specific alterations in oral microbiota and those more generally related to poor oral health [21]. Also, decreased salivary flow because of medication can alter environmental characteristics, such as acid concentration and bacterial adhesion to surfaces, subsequently altering oral microbiota composition [22]. Finally, oral dysbiosis can trigger various immunological mechanisms that promote disease onset and progression, further contributing to imbalances in the gut microbiota [23]. This bidirectional relationship makes it challenging to distinguish between the cause and effect of observed microbiota alterations.

Although asthma and its treatments have been associated with altered saliva flow and pH and oral health parameters, the mechanisms underlying these associations remain poorly understood. Previous studies have primarily focused on cariogenic bacteria such as *S. mutans* or on general oral hygiene parameters, often without including healthy controls or microbiome profiling. In particular, few studies have evaluated the broader composition and diversity of the salivary microbiota in children with asthma or linked these changes to clinical oral health outcomes [20].

Therefore, the aim of this study was to investigate the association between asthma, anti-asthmatic therapy, and oral health parameters in children, and to explore whether alterations in salivary microbiota might mediate these effects. Understanding these relationships could help optimize preventive dental care in children with asthma and guide the development of safer therapeutic strategies.

## Materials and methods

### Participants and study design

We conducted a cross-sectional study that included all children with asthma treated in Paediatric pulmonology outpatient clinic at our University teaching hospital between 1 October 2022 and 29 February 2024. Children aged 6 to 18 years at the time of assessment were eligible for inclusion. The diagnosis of asthma was established by a paediatric pulmonologist according to the European Respiratory Society (ERS) criteria. Diagnosis required either a positive bronchodilator reversibility test, defined as an increase in forced expiratory volume in one second ($FEV_1$) of at least 12% and 200 mL following administration of 4 puffs of SABA (salbutamol), and/or a highly positive bronchoprovocation test with methacholine, indicated by a 20% decrease in $FEV_1$ at a cumulative methacholine dose (PD20) of less than 0.4 mg [24]. Asthma duration was determined based on parental history, defined from the first wheezing episode occurring after the child's third year of age.

Exclusion criteria included children with other chronic lung diseases (e.g., cystic fibrosis, primary ciliary dyskinesia, chronic aspiration) or immune-mediated diseases (e.g., autoimmune diseases, inflammatory bowel diseases, immunodeficiencies, celiac disease). Additional exclusions encompassed renal failure, chronic neurological or neuromuscular diseases (including epilepsy), global developmental delay, swallowing disorders, metabolic or systemic skeletal disorders, autism spectrum disorders, feeding disorders, conditions affecting oral health, and children born before 32 weeks of gestation. We also excluded children taking immunosuppressive drugs (other than ICS) or medications affecting oral health and/or the oral microbiota (e.g., antibiotics or probiotics) within the last month prior to the assessment. Children with allergic conditions, such as allergic rhinitis or atopic dermatitis were not excluded.

Asthma control was assessed using the Asthma Control Test (ACT) questionnaire, and the ACT score was recorded [25]. The daily ICS dose (standardized to budesonide equivalents) [3] over the preceding six months, as well as the use of LABA and/or SABA within the last week before assessment, were documented.

As a control group, we included 50 healthy children (without acute or chronic disease and not taking any medication), matched for sex and age to the children with asthma. The sample size calculation for this study was based on a two-sample t-test to evaluate the difference in DMFT/dft index between children with asthma and healthy controls. A statistical power of 0.82 is achieved with 100 participants in the experimental group and 50 in the control group, assuming a population standard deviation of 2 and a clinically relevant difference of 1, using a two-sided significance level of 0.05. The sample size was calculated using Clin.Calc [26].

All patient data were anonymized prior to statistical analysis.

#### Evaluation of pulmonary function and allergy tests

Spirometry, a methacholine bronchoprovocation test, and fractional exhaled nitric oxide (FeNO) measurements were performed using a Ganshorn SpiroScout spirometer (Ganshorn Medizin Electronic GmbH, Niederlauer, Germany), adhering to American Thoracic Society (ATS) and ERS guidelines [24, 27]. $FEV_1$, recorded as a percentage of the reference value [28], $FEV_1$/forced vital capacity (FVC) ratio (Tiffeneau index, TI), and FeNO values (in parts per billion, ppb) were recorded. Allergic asthma was considered in patients sensitized to airborne allergens, exhibiting symptoms triggered by allergen exposure, and a FeNO value > 25 ppb, indicative of eosinophilic airway inflammation [24]. Most patients also underwent a methacholine bronchoprovocation test, with the PD20 calculated and documented.

Hypersensitivity to common airborne allergens was assessed using skin prick tests (Lofarma, Milan, Italy) and/or by measuring specific immunoglobulin E serum levels (sIgE) via the ImmunoCAP system (Phadia, Uppsala, Sweden). Hypersensitivity was defined as a skin induration at least 3 mm larger than the negative control and/or sIgE level > 0.35 IU/ml. Total sIgE were also determined.

#### Dental examination and saliva analysis

All dental examinations were performed by a single paediatric dentist with three years of specialized training in pediatric oral health. An oral hygiene and diet questionnaire was completed concurrently (see Supplementary material 1). Oral health data were recorded in a specialized dental record aligned with World Health Organization (WHO) guidelines [29]. Metrics included the DMFT index (Decayed, Missing, and Filled Teeth) for permanent teeth and the related dft (decayed and filled teeth) index for deciduous teeth. Although the DMFT and dft indices represent different dentitions, a combined caries index (DMFT/dft) was used as an indicator of total caries burden for the purpose of statistical analysis [30]. Additional metrics included dental erosion score (assessed using the Basic Erosive Wear Examination (BEWE) system) [31], the Simplified Oral Hygiene Index (OHI-S) [32], and gingival inflammation scores (Löe&Silness gingival index) [33].

Saliva samples were collected between 9:00–10:00 AM. Participants refrained from eating, drinking, or brushing their teeth for at least two hours prior to sampling. Unstimulated saliva was collected over a three-minute period using a sterile plastic syringe, with the volume recorded [34], and then analyzed for pH using SimplexHealth test strips (SimplexHealth, Wollaston, UK). Saliva samples designated for sequencing were transferred to the collaborating laboratory of the day of collection at room temperature and subsequently stored at −80 °C until further processing.

#### 16 S rRNA amplicon sequencing

Total DNA was isolated from saliva samples using the commercial MasterPure Complete DNA and RNA Purification Kit (LGC Biosearch Technologies, London, England). 16 S amplicon sequencing was performed by targeting variable region V3-V4 of the 16 S rRNA gene using the primer pair Bakt_341F (5'-CCTACGGGNGGCWGCAG-3')-Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3') [35]. Sequencing was performed on Illumina NextSeq(2 × 300 bp). Raw sequence reads were quality filtered and assigned to amplicon sequencing variants (ASVs) implementing mothur (v.1.48.0); the taxonomy was inferred based on RDP database (trainset v.9)

[36]. Sequencing data is accessible in the NCBI database under accession number PRJNA1196312.

Statistical analysis and visualization of the 16 S amplicon sequencing results were performed in R software environment for statistical computing and graphics using the packages ‚vegan' and 'ggplot'. Analysis of molecular variance (AMOVA) and analysis of similarity (ANOSIM) statistical tests were used to assess differences in microbial structure between children with asthma and controls. Permutational multivariate analysis of variance (PERMANOVA) was used to assess the association of asthma phenotypic characteristics with microbial structure. For identification of differentially abundant bacterial taxa, LEfSe test implemented in mothur was used. The Wilcoxon rank sum test was used to compare the alpha diversity of salivary microbiota composition between children with asthma and controls, and Pearson's r correlation coefficient was used to assess the correlation between quantitative phenotypic characteristics of children with asthma and their salivary microbiota diversity. The Benjamini-Hochberg procedure was applied to adjust p-values for multiple comparisons, controlling the false discovery rate (FDR) at a threshold of 0.05.

### Statistical analysis

Statistical analysis was performed using IBM SPSS 29.0 (IBM Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test assessed normality. Mann-Whitney U tests compared continuous or ordinal variables between groups. Spearman's rank correlation coefficient analyzed relationships between oral health parameters and asthma phenotypes. Statistical analysis was extended using generalized linear models (with a binomial distribution) adjusted for potential confounders (e.g., age, sex). A p-value < 0.05 was considered statistically significant.

## Results

### Demographic and clinical characteristics of children with asthma

We included 100 children with asthma and 50 controls (38% female vs. 62% male, $p = 0.86$). Patients and controls were matched in their responses to a questionnaire on oral hygiene and diet (see Supplementary material 1). The median age was 12 years (IQR = 6) in both children with asthma and controls, and the mean age was 12 years in both groups as well (SD = 3.5 in children with asthma and 3.4 in controls; $p = 1.00$). The allergic asthma phenotype was diagnosed in 86 (86%) children. Sixty-one (61%) received daily ICS, 41 (67%) in combination with LABA. The median ICS dose was 160 mcg (IQR = 100 mcg) of budesonide equivalent. In the week before the evaluation, 49 (49%) children with asthma used reliever medications.

### Oral health in children with asthma

The parameters of oral health in children with asthma compared to healthy controls are summarized in Table 1.

The mean DMFT/dft was 1.13 (SD = 2.12) in children with asthma and 0.28 (SD = 0.88) in controls ($p < 0.01$). Only 5 children with asthma had dental erosions, and all were minimal (BEWE score = 1), while no erosions were found in the control group ($p = 0.08$). There were no significant differences in oral health parameters, salivary secretion and pH between males and females, between allergic and non-allergic children with asthma, and between children with asthma treated daily with ICS as monotherapy or in fixed combination with LABA.

All healthy controls were found to have normal gingiva (gingival index = 0) compared to the group of children with asthma, where only 4 (4%) children had normal gingiva, 55 (55%) had mild inflammation (gingival index = 1) and 41 (41%) had moderate inflammation (gingival index = 2), $p < 0.01$.

**Table 1** Comparison of oral health parameters between children with asthma and controls

| Parameter (Median IQR) | Controls (*N* = 50) | Children with asthma (*N* = 100) | *p*-value[1] | Regular daily therapy[2] | | *p*-value[3] | Reliever therapy[4] | | *p*-value[5] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | YES (*N* = 61) | NO (*N* = 39) | | YES (*N* = 49) | NO (*N* = 51) | |
| DMFT/dft index | 0 (2) | 4 (6) | **< 0.01** | 4 (6) | 4 (6) | 0.75 | 4 (6) | 4 (5) | 0.70 |
| OHI–S | 0 (0) | 0.9 (0) | **< 0.01** | 1 (0) | 0.8 (0) | **< 0.01** | 0.9 (0) | 0.8 (0) | 0.29 |
| Gingival index[6] | 0 (0) | 1 (1) | **< 0.01** | 1 (1) | 1 (1) | 0.78 | 2 (1) | 1 (1) | **0.02** |
| Amount of saliva (ml) | 2.5 (0) | 1.3 (1.0) | **< 0.01** | 1 (1.0) | 1.5 (0) | **< 0.01** | 1.4 (1) | 1.3 (1) | 0.92 |
| pH of saliva | 7.00 (0.25) | 6.25 (0.75) | **< 0.01** | 6.25 (0.50) | 6.75 (0.50) | **< 0.01** | 6.00 (1.00) | 6.25 (0.75) | **0.01** |

IQR interquartile range, DMFT/dft sum of decayed, missing and filled permanent and/or deciduous teeth, OHI-S simplified oral hygiene index (assessment of dental plaque and calculus) [26]

[1] p refers to the comparison between children with asthma and controls

[2] regular daily treatment with inhaled corticosteroids with or without long-acting beta2-agonist in a fixed combination, over the last 6 months

[3] p refers to the comparison between children with asthma receiving and not receiving regular daily therapy

[4] uses of short-acting or long-acting beta2-agonists as reliever medication in the last week

[5] p refers to the comparison between children with asthma who used reliever therapy in the last week and those who did not

[6] scoring of gingival inflammation according to the Löe-Silness method [27]

The correlation analysis between oral health parameters and quantitative epidemiological and clinical characteristics of children with asthma is summarized in Table 2.

The results were further analyzed using multiple linear regression, adjusting for sex and age, and we still found a higher DMFT/dft index in children with asthma highly significant (R2 = 0.33, $p < 0.01$).

### Analysis of salivary microbiota

To further explore the microbiological component of these findings, salivary microbiota composition was analyzed. The salivary bacterial community structure differed significantly between children with asthma and healthy controls (AMOVA, $p = 0.001$; ANOSIM, $p = 0.03$; Fig. 1A). On average, approximately 160 bacterial taxa were detected per sample. Both community richness (Chao richness, $p < 0.01$) and evenness (Shannon evenness, $p = 0.01$) were significantly higher in healthy controls compared to children with asthma. However, the absolute differences were small (cases-associated 5% and 3% decrease in community richness and evenness, respectively) (Fig. 1B).

The salivary microbiota was dominated by five phyla (Fig. 1C), with a total of 21 phyla detected at a minimum threshold of 10 sequencing reads. No significant differences were observed between cases and controls at the phylum level. However, several genera were found to be differentially abundant (Fig. 1C, all listed genera displayed significant differential abundance after FDR < 0.05). A statistically significant increase in *Streptococcus*, *Gemella*, and *Streptobacillus* was observed in cases. Within *Streptococcus*, which was represented by 31 distinct amplicon sequence variants (ASVs), individual ASVs exhibited variable dynamics despite the overall increase in genus in cases. Two ASVs demonstrated statistically significant differential abundance: ASV1 increased and ASV30 decreased in cases compared to controls (LEfSe, LDA scores of 4.35 and 3.29 for ASV1 and ASV30, respectively; FDR < 0.05). In contrast, controls showed an increase in 11 genera, consistent with the higher overall richness observed in alpha diversity analysis (Fig. 1B). Among these, *Actinomyces*, *Oribacterium*, *Campylobacter*, and *Atopobium* were significantly enriched (Fig. 1C).

Permutational multivariate analysis of variance (PERMANOVA) conducted on cases alone revealed no association between bacterial community structure and demographic data (age, sex), oral health indicators (OHI-S, gingival index, DMFT/dft index), or disease-related factors such as duration, treatment, or asthma type (allergic vs. non-allergic). Together, all metadata included in PERMANOVA test explained less than 10% of between-sample variability, and none reached the significance threshold of 0.05 (Table 3).

**Table 2** Correlations analysis between oral health parameters and quantitative clinical characteristics of children with asthma

| Characteristic (Spearman ρ, (*p*-value)) | DMFT/dft index | OHI-S | Gingival index[1] | Amount of saliva | pH of saliva |
|---|---|---|---|---|---|
| Age | 0.01 ($p = 0.94$) | 0.41 ($p = 0.62$) | 0.06 ($p = 0.50$) | 0.07 ($p = 0.40$) | 0.03 ($p = 0.72$) |
| Duration of asthma | −0.07 ($p = 0.94$) | 0.37 (***p* < 0.01**) | −0.01 ($p = 0.94$) | −0.42 (***p* < 0.01**) | 0.14 ($p = 0.17$) |
| $FEV_1$ | 0.19 ($p = 0.06$) | −0.05 ($p = 0.66$) | 0.05 ($p = 0.62$) | 0.10 ($p = 0.34$) | 0.12 ($p = 0.22$) |
| TI | 0.14 ($p = 0.18$) | −0.09 ($p = 0.39$) | −0.05 ($p = 0.64$) | 0.05 ($p = 0.65$) | 0.10 ($p = 0.34$) |
| PD20 | −0.12 ($p = 0.30$) | −0.14 ($p = 0.23$) | 0.11 ($p = 0.36$) | 0.02 ($p = 0.90$) | 0.15 ($p = 0.19$) |
| FeNO | 0.01 ($p = 0.89$) | 0.06 ($p = 0.55$) | −0.07 ($p = 0.47$) | 0.04 ($p = 0.68$) | 0.12 ($p = 0.24$) |
| ACT score | 0.10 ($p = 0.31$) | −0.04 ($p = 0.71$) | −0.10 ($p = 0.32$) | 0.02 ($p = 0.83$) | 0.36 (***p* = 0.01**) |
| Total serum IgE | 0.17 ($p = 0.14$) | 0.06 ($p = 0.61$) | 0.22 (***p* = 0.05**) | −0.13 ($p = 0.27$) | 0.04 ($p = 0.74$) |
| Daily ICS dose | 0.17 ($p = 0.20$) | 0.01 ($p = 0.95$) | 0.01 ($p = 0.96$) | −0.08 ($p = 0.55$) | 0.26 (***p* = 0.05**) |

$FEV_1$ forced expiratory volume in one second as a percentage of the predicted reference value, TI Tiffeneau index, representing the ratio between $FEV_1$ and forced vital capacity, PD20 cumulative provocation dose of methacholine required to cause 20% reduction in $FEV_1$ from baseline, FeNO fractional exhaled nitric oxide. ppb part per billion, ACT Asthma Control Test [22], DMFT/dft sum of decayed, missing and filled permanent and/or deciduous teeth, OHI-S simplified oral hygiene index (assessing dental plaque and calculus) [26]

[1] scoring of gingival inflammation according to the Löe-Silness method [27]

## Discussion

This study demonstrates that children with asthma exhibit significantly poorer oral health compared to healthy controls, with worse indices for dental caries (DMFT/dft index: median 4 vs. 0), dental plaque and calculus (OHI-S), and gingivitis (gingival index). These findings align with meta-analyses, which reported higher caries prevalence in children (in permanent and primary teeth) and adults with asthma [37, 38].

The association between asthma and oral health issues is often attributed to long-term ICS use, which reduces salivary pH and promotes growth of cariogenic microbiota representatives [39]. Additionally, different lifestyle of children with asthma (diet, reduced oral hygiene) could further promote the development of caries [40, 41]. However, in our study, daily ICS therapy did not significantly increase the DMFT/dft index compared to non-ICS users, nor was there a correlation between ICS dose and caries or salivary microbiota composition. Instead, ICS users had higher plaque and calculus index scores, a known risk factor for caries [42], lower salivary pH and volume. Furthermore, higher plaque index was correlated with longer asthma duration. These findings are

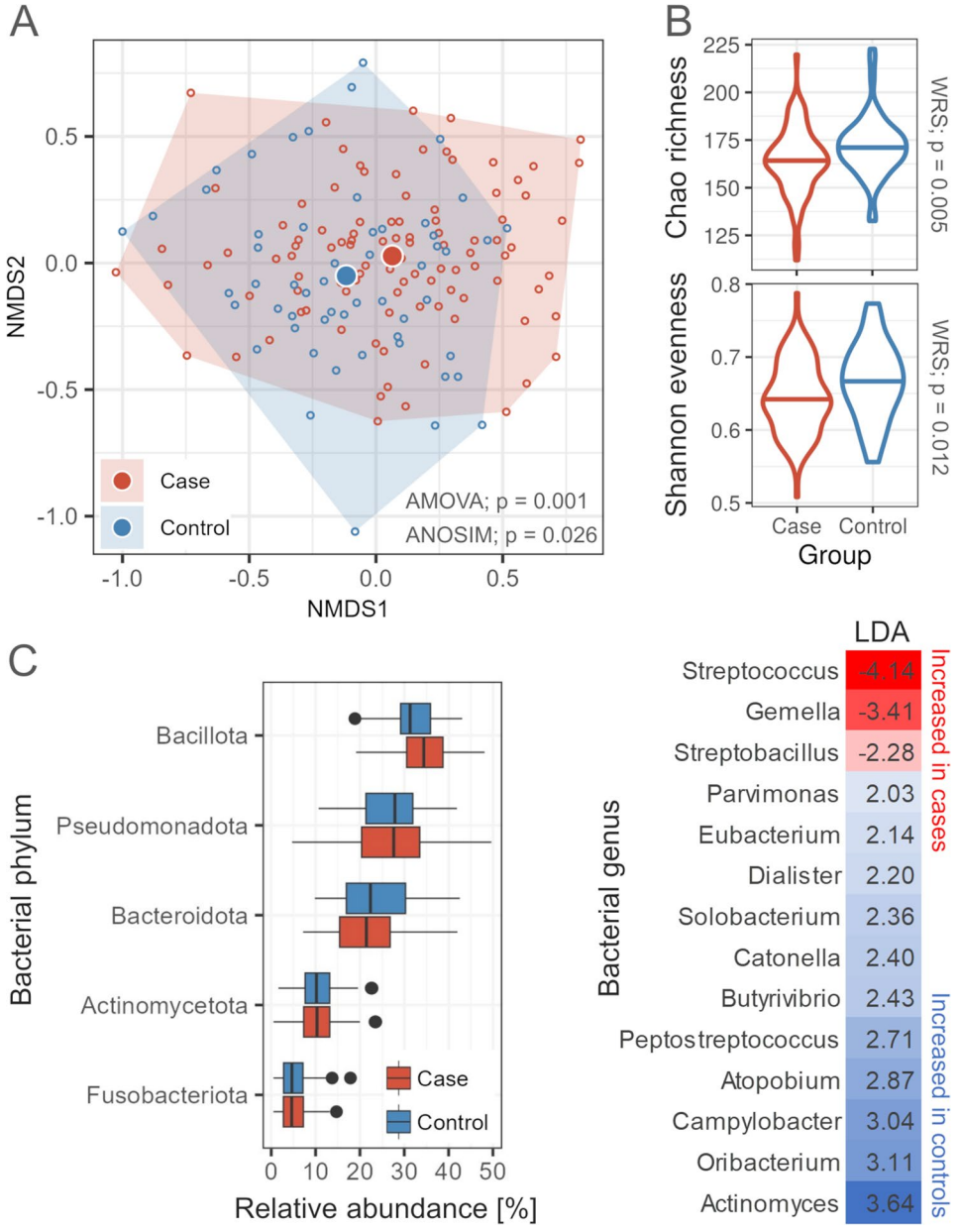


**Fig. 1** Salivary bacterial community analysis. **A** Beta diversity analysis visualized using NMDS, with cases shown in red and controls in blue. **B** Alpha diversity analysis illustrating bacterial community richness (Chao index) and evenness (Shannon index). **C** Population-level analysis at the taxonomic levels of bacterial phylum (left) and genus (right). Differentially abundant genera are highlighted with corresponding linear discriminant analysis (LDA) values based on the LEfSe test. All genera displayed are statistically significant at a false discovery rate (FDR), $p < 0.05$

consistent with those of other researchers, suggesting that ICS indirectly affects oral health by altering salivary pH and volume, thereby facilitating dental plaque formation [43, 44]. The observed reduction in salivary flow in our patients, correlated with asthma duration and associated with regular ICS therapy in our study, is likely one of the most significant pro-cariogenic factors in children with asthma. This finding, along with the strong negative correlation between the DMFT/dft index and salivary flow (Spearman's ρ=−0.5, $p < 0.01$; data not shown), is consistent with previous research [5]. The absence of an effect of beta-2 agonists on salivary flow in our study may be partially explained by the fact that most children with asthma received only low doses of LABA as part of daily controller therapy with a fixed ICS/LABA combination. Moreover, asthma was well controlled in the vast majority of participants, and no significant exacerbations occurred during the week preceding the assessment that would have required intensive beta-2 agonist treatment.

**Table 3** Association between children with asthma characteristics and salivary bacterial community structure

| Characteristic (PERMANOVA, *p*-value) | Explained variance (R2) | *p*-value[1] |
|---|---|---|
| Age (years) | 0.009 | 0.626 |
| Sex | 0.010 | 0.462 |
| DMFT/dft index | 0.012 | 0.214 |
| OHI-S | 0.010 | 0.492 |
| Saliva (mL) | 0.005 | 0.995 |
| Saliva pH | 0.013 | 0.172 |
| Duration of asthma (years) | 0.009 | 0.636 |
| Regular daily therapy (Yes/No) | 0.010 | 0.490 |
| Reliever therapy (Yes/No) | 0.007 | 0.837 |
| Allergic asthma (Yes/No) | 0.009 | 0.613 |

DMFT/dft sum of decayed, missing and filled permanent and/or deciduous teeth, OHI-S simplified oral hygiene index (assessment of dental plaque and calculus) [26].

[1] p-value refers to the results of permutational multivariate analysis of variance.

Beta-2 agonists (SABA and LABA), used as reliever medication for asthma symptoms, were not found to significantly affect salivary secretion, which contrasts with previous studies suggesting both ICS and beta-2 agonists reduce saliva flow and promote dental caries [45, 46]. Nonetheless, we observed reduced salivary pH with beta-2 agonist use (as reliever), consistent with prior findings [44]. Improved asthma control, reflected by higher ACT scores, was correlated with higher salivary pH in our study, which may reflect a reduced reliever therapy usage (SABA or LABA) in children with better-controlled asthma. Interestingly, our study found no significant difference in salivary pH between children with asthma receiving continuous daily ICS monotherapy and those treated with a fixed ICS/LABA combination. However, a reduction in salivary pH was observed with LABA use as a reliever. This may reflect higher cumulative doses of LABA typically used during exacerbations, or it may suggest that asthma exacerbations themselves, independent of treatment, influence salivary pH through other underlying mechanisms.

Children with asthma also exhibited significantly higher gingival indices, as already reported previously, but in correlation with lower $FEV_1$ [47]. In our study, 96% showed mild gingival inflammation, compared to normal gingiva observed in all healthy controls. However, we found no association between asthma severity (lower $FEV_1$ and/or ACT score) or allergic inflammation markers (e.g., total IgE, FeNO) and oral health, except for a marginally significant positive correlation between total IgE with the gingival index. Our results, therefore, do not support the view that poor oral health in children with asthma is attributable to allergic rhinitis and mouth breathing [48]. Instead, these findings are more consistent with previous reports that allergic airway inflammation may not be a key contributor to dental caries [49].

Anti-asthmatic therapy has evolved over the past two decades, particularly with the increased use of fixed combinations of ICS and LABA delivered via a single inhaler device. Initially reserved as a daily controller therapy for moderate to severe persistent asthma in adults, fixed ICS/LABA combinations have been more widely recommended since the 2019 GINA guidelines.

Fixed ICS/LABA combinations are now endorsed as first-line daily controller therapy for children aged 5 to 18 years with moderate to severe asthma, and for intermittent use (administered only when symptoms occur) in children over 12 years with mild asthma. In children under 12 years of age, ICS monotherapy remains the cornerstone of treatment for mild persistent asthma [3]. However, in our study, we found no significant differences in oral health parameters between children treated daily with ICS monotherapy and those daily receiving a fixed ICS/LABA combination therapy. Non-carious dental lesions, such as erosive tooth wear, are increasingly recognized as important indicators of oral health. However, the prevalence of erosive tooth wear in our study was lower than anticipated, 5% among children with asthma and 0% among healthy controls, thereby limiting its usefulness for associational analyses. This unexpectedly low incidence may reflect the subjective nature of the Basic Erosive Wear Examination (BEWE), particularly with respect to score 1, which denotes initial surface texture loss. Therefore, alternative indices may be more suitable for assessing erosive tooth wear in paediatric populations [31, 50]. The analysis of salivary microbiota revealed modest yet significant differences between children with asthma and healthy controls. Asthmatic individuals showed increased abundance of *Streptococcus*, *Gemella*, and *Streptobacillus*. Genus *Streptococcus* comprises a diverse community with roles ranging from beneficial core microbial community assembly, immune modulation and colonization resistance [51] to adverse, most notably cariogenic (*S. mutans and S. sobrinus*) [52]. While we did observe diverse dynamics of individual *Streptococcus* ASVs, the method used in this study does not allow reliable species-level classification. *Gemella* and *Streptobacillus* are not typically linked to caries [53], furthermore *Gemella haemolysans* may protect against periodontal disease by inhibiting pathogenic bacteria [54]. However, this species can exhibit higher abundance in children predisposed to allergic diseases [55]. The increased abundance of *G. haemolysans* in our children with asthma is therefore unlikely to reflect a direct etiological role in caries development but may instead be related to its known association with the pathogenesis of allergy and asthma [56].

Higher diversity in the gut microbiota is often associated with health, but its role in oral microbiota remains less clear. The reduced diversity observed here in children

with asthma is consistent with reports of lower microbial diversity in allergic diseases and dental caries [55, 57]. Conversely, other studies have described greater oral microbial diversity in children with asthma and comorbid allergic rhinitis, whereas no differences were observed in those without allergic rhinitis [18]. In our study, higher diversity was associated with increase in several genera, most prominently *Actinomyces*, *Oribacterium*, and *Atopobium*. These genera were previously found to be inhibited by streptococci like *Streptococcus dentisani* [58], potentially explaining their decrease in asthmatic patients. Finally, we found no associations between salivary microbiota, oral health indices, or asthma-related parameters, diverging from studies linking specific taxa, such as *S. mutans* and *Lactobacillus*, to ICS use and caries [7, 59].

Overall, differences in microbiota composition between asthmatic patients and the healthy population were statistically significant but of small effect size, warranting further research to elucidate their potential biological relevance. Studies involving larger cohorts, site-specific sampling (e.g., dental plaque) and the integration of functional analyses (such as metabolomics) alongside host factors may provide a more comprehensive understanding of these interactions and better account for population variability.

### Limitations

The study's limitations include heterogeneity in asthma treatment regimens, variability in SABA/LABA dosing during exacerbations, and reliance on saliva samples, which provide a composite view of the oral microbiota but lack niche specificity. The use of combined DMFT/dft index may have obscured differences between primary and permanent dentition. In addition, the control group comprised only half as many samples as the test group, which may have influenced comparisons due to unbalanced confounding factors.

Moreover, 16 S amplicon sequencing did not allow species-level resolution, limiting insights into health-associated and pathogenic species (*S. Mutans*) within detected genera. In addition, cross-sectional study design precluded causal inference between microbiota and confounding factors. Finally, the sensitivity of the applied methods to detect associations between oral health indices, anti-asthmatic therapy, and the salivary microbiota may have been limited by the exclusion of initial caries (i.e., enamel demineralisation without cavitation) [6]. However, incorporating initial caries would compromise the objectivity of our dental assessments, as such lesions are not accounted for in the standard DMFT/dft index.

## Conclusions

Asthma appears to be associated with an increased risk of poor oral health in children, increasing susceptibility to caries and periodontal disease. The worse oral health indices in children with asthma were not fully explained by anti-asthmatic therapy, asthma severity, or salivary microbiota. Despite these findings, meticulous oral hygiene remains crucial for children with asthma.

#### Abbreviations

| | |
|---|---|
| ACT | Asthma Control Test |
| ATS | American Thoracic Society |
| BEWE | Basic Erosive Wear Examination |
| DMFT/dmf | sum of decayed, missing and filled permanent and/or decidous teeth |
| ERS | European Respiratory Society |
| $FEV_1$ | Forced expiratory volume in one second as a percentage of the predicted reference value |
| FeNO | Fractional exhaled nitric oxide |
| FVC | Forced vital capacity |
| ICS | Inhaled corticosteroids |
| LABA | Long-acting beta-2 agonists |
| OHI-S | Simplified Oral Hygiene Index |
| ppb | part per billion |
| SABA | Short-acting beta-2 agonists |
| TI | Tiffeneau index |
| WHO | World Health Organization |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12887-025-06485-9.

Supplementary Material 1.

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Not applicable.

#### Authors' contributions

Conceptualization, VB, BE, APL, MR, AM; investigation and data curation, VB, BE, AM; methodology, VB, AM; formal analysis, VB, AM; software AM; validation, VB, MR, AM, project administration VB, MR, resources, VB, MR. BE; supervision, VB, MR; writing - original draft, VB, APL, AM; writing - review & editing, VB, APL. All authors have read and agreed to the published version of the manuscript.

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#### Data availability

The data that support the fundings of this study are openly available in Kaagle, at DOI: https://doi.org/10.34740/kaggle/dsv/10918637.

## Declarations

#### Ethics approval and consent to participate

The study was approved by the National Medical Ethics Committee of the Republic Slovenia (0120 – 99/2022/4, 5 May 2022) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in Edinburgh in 2000. Informed consent was obtained from all participants or their guardians (for children under 15 years).

**Consent for publication**
Patients or their caregivers (for children under 16 years of age) provided an informed consent for the anonymized publication of the results.

**Competing interests**
The authors declare no competing interests.

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