Association of corticosteroid inhaler type with saliva microbiome in moderate-to-severe pediatric asthma

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Abstract

Association of corticosteroid inhaler type with saliva microbiome in moderate-to-severe pediatric asthma Background Metered dose inhalers (MDIs) and dry powder inhalers (DPIs) are common inhaled corticosteroid (ICS) inhaler devices. The difference in formulation and administration technique of these devices may influence oral cavity microbiota composition. We aimed to compare the saliva microbiome in children with moderate-to-severe asthma using ICS via MDIs versus DPIs. **Methods** Saliva samples collected from 143 children (6-17 yrs) with moderate-to-severe asthma across four European countries (the Netherlands, Germany, Spain, and Slovenia) as part of the SysPharmPediA cohort were subjected to 16S rRNA sequencing. Microbiome was compared using global diversity (α and β) between two groups of participants based on inhaler devices (MDI (n=77) and DPI (n=65)) and differential abundance was compared using the Analysis of Compositions of Microbiomes with the Bias Correction (ANCOM-BC) method. **Results** No significant difference was observed in α-diversity between the two groups. However, β-diversity analysis revealed significant differences between groups using both Bray-Curtis and weighted UniFrac methods (Adjusted p-value=0.015 and 0.044, respectively). Significant differential abundance between groups, with higher relative abundance in the MDI group compared to the DPI group, was detected at the family level [Carnobacteriaceae (Adjusted p=0.033)] and at the genus level [*Granulicatella* (Adjusted p=0.021) and *Aggregatibacter* (Adjusted p=0.011)]. **Conclusion** Types of ICS devices are associated with different saliva microbiome composition in moderate-to-severe pediatric asthma. The causal relation between inhaler types and changes in saliva microbiota composition needs to be further evaluated, as well as whether this leads to different potential adverse effects in terms of occurrence and level of severity.

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Abstract

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Background

Metered dose inhalers (MDIs) and dry powder inhalers (DPIs) are common inhaled corticosteroid (ICS) inhaler devices. The difference in formulation and administration technique of these devices may influence oral cavity microbiota composition. We aimed to compare the saliva microbiome in children with moderate-to-severe asthma using ICS via MDIs versus DPIs.

Methods

Saliva samples collected from 143 children (6-17 yrs) with moderate-to-severe asthma across four European countries (the Netherlands, Germany, Spain, and Slovenia) as part of the SysPharmPediA cohort were subjected to 16S rRNA sequencing. Microbiome was compared using global diversity (α and β) between two groups of participants based on inhaler devices (MDI (n=77) and DPI (n=65)) and differential abundance was compared using the Analysis of Compositions of Microbiomes with the Bias Correction (ANCOM-BC) method.

Results

No significant difference was observed in α-diversity between the two groups. However, β-diversity analysis revealed significant differences between groups using both Bray-Curtis and weighted UniFrac methods (Adjusted p-value=0.015 and 0.044, respectively). Significant differential abundance between groups, with higher relative abundance in the MDI group compared to the DPI group, was detected at the family level [Carnobacteriaceae (Adjusted p=0.033)] and at the genus level [*Granulicatella* (Adjusted p=0.021) and *Aggregatibacter* (Adjusted p=0.011)].

Conclusion

Types of ICS devices are associated with different saliva microbiome composition in moderate-to-severe pediatric asthma. The causal relation between inhaler types and changes in saliva microbiota composition needs to be further evaluated, as well as whether this leads to different potential adverse effects in terms of occurrence and level of severity.

Keywords: Asthma, Inhaled Corticosteroids, Metered Dose Inhaler, Dry Powder Inhaler, Microbiome, Saliva

Key Message:

The type of inhaled corticosteroid (ICS) devices (metered dose inhaler (MDI) versus dry powder inhaler (DPI)) is associated with different saliva microbiome composition in moderate-to-severe pediatric asthma. The findings suggest that inhaler device types should be recognized as potential confounding factors in asthma oral microbiome studies. Furthermore, the results shed light on the potential impact of these devices on the oral microbiota composition, which in turn may affect the frequency and intensity of oral health-related adverse effects.

Introduction

Inhaled corticosteroids (ICS) are among the main choices in managing and treating asthma and are recommended for mild-to-severe asthma in children and adults. [1,2] It is essential to consider active molecules, patients' preferences, and user abilities while prescribing an ICS for asthma. Metered dose inhalers (MDIs) and dry powder inhalers (DPIs) are common ICS inhaler devices.[3,4] MDI inhalers are well-known, compact, convenient devices requiring actuation coordination to be used correctly. The breath and actuation coordination sometimes makes using the MDI inhalers difficult for some patients with low cognitive ability or difficulty actuating. MDI can be used with a spacer to improve medication delivery by extending the time of inhaling and allowing the lungs to absorb the medication more slowly and smoothly. In addition, using a spacer simplifies the inhaling process by reducing the need for actuation coordination in MDI users, especially in children. [5]MDI inhalers are cheaper than DPIs, and propellants are essential for their formulation. Breath-dose coordination is unnecessary while using DPI inhalers because DPIs are designed to release the medication in response to the patient's inhalation effort. However, sufficient respiratory force is required for DPI inhalers to inhale the powder effectively. User instruction is inconsistent among different DPI inhalers, and a preparation step is usually needed before inhalation.[3,4,6,7]

The airway microbiome has been associated with the development and severity of asthma. [6,8] It was shown that bacterial diversity and enriched pathogenic species are higher in the airways of asthma patients compared to healthy individuals. [8,9]The lung microbiome is partly derived from the upper respiratory tract, as well as its composition is being influenced due to interactions with the oropharynx microbiome. [10] Hence, saliva may be a more feasible and completely non-invasive source for microbiome studies, especially in children where other samples like induced sputum, biopsies, and bronchoalveolar collection are challenging and invasive.[11] The saliva microbiome is in direct contact with both airway and gastrointestinal (GI) entries. Different environmental factors may influence the saliva microbiome, like food, drinks, and medications. It has been previously shown that ICS treatment is associated with the airway microbiome composition and diversity.[12,13] However, more research is needed to better understand the association between inhaled corticosteroid intake

and salivary microbiome in children to optimize risk assessment, monitoring, and management strategies to protect patients' oral and overall health while using inhaled corticosteroids.

Local deposition of ICS in the oropharynx and larynx may lead to side effects like dysphonia (hoarse voice), and oropharyngeal candidiasis (thrush). The severity and frequency of side effects depend on medication type, dose, administration rate, inhaler techniques, and device type, which can be prevented by rinsing the mouth and throat with water after using all ICS types. [14-16] The risk of oral candidiasis and dysphonia was higher by using MDI devices than DPI devices when compared to a placebo group.[17] We hypothesized that differences in formulation and ICS device types may influence oral cavity microbiota composition. This study aims to compare the saliva bacterial microbiome in children with moderate-to-severe asthma under regular ICS treatment via MDI versus DPI devices.

Methods

Study design

The SysPharmPediA study is a multicenter European observational study with a case-control setting. The study design and study population were described previously in detail by Abdel-Aziz et al. [18]Briefly, children aged 6-17 with doctor-diagnosed moderate-to-severe asthma were included at tertiary hospitals from four European countries: the Netherlands, Germany, Spain, and Slovenia. This study with registration identifier NCT04865575 (at www.clinicaltrials.gov) was carried out based on the Helsinki Declaration and approved by the medical ethics committee of the University Medical Center Utrecht, the Netherlands (NL55788.041.15); National Medical Ethics Committee, Slovenia (0120–569/2017/4), the ethics committee of University Regensburg, Germany (18–1034–101); Clinical Research Ethics Committee of the Basque Country, Spain (PI2015075); All participants or their caretaker or parents gave their informed consent.

Study Population

Saliva samples (n=143) were collected from the SysPharmPediA participants (Figure 1). Clinical evaluation, doctor-reported medication use, inhaler device types, medication adherence, and inhaler techniques assessments were conducted at the inclusion time, which was previously described in detail. [18,19]

Saliva sample collection, 16S rRNA sequencing and processing

A detailed description of saliva sample collection, 16S rRNA isolation, sequencing, and read processing was reported previously.[20] In summary, a total of 143 saliva samples were collected at the inclusion time in falcon tubes and stored at -80°C in each center until the shipment to a long-term storage biobank in Regensburg, Germany. Polymerase chain reaction (PCR) amplification using specific primers (hypervariable V3-V4 selective of 16S rRNA) was performed. Based on standard Illumina protocol, samples were sequenced by MiSeq V3 2×300 bp. Sequencing of more than 10,000 reads per sample was considered. After the quality control using MultiQC,[21] and FastQC, [22] primers were removed by Cutadapt, [23] and then the DADA2 pipeline,[24] was followed (GitHub: https://benjjneb.github.io/dada2/tutorial.html). Furthermore, according to the SILVA database v138, [25] amplicon sequence variants (ASVs) were annotated to their respective bacterial taxonomy.

Statistical analysis

Visualization techniques (Q-Q plots and histograms) and Kolmogorov-Smirnov tests were applied to assess the distribution of continuous variables. Mean ± standard deviation (SD) and median and 25^{th} and 75^{th} percentiles were reported for continuous variables that followed normal and non-normal distributions, respectively. Demographic and clinical characteristics of DPI and MDI device users were compared using parametric and nonparametric tests (Mann-Whitney U test, Pearson Chi-Square, or Fisher's Exact tests). According to the literature and consulting with experts in the team, confounding factors were drawn in the directed acyclic graph (DAG, Figure 2) and, if applicable, considered for adjustment in further analyses. Bacterial microbiome composition and global diversity (α and β diversity using phyloseq, [26]and vegan [27] R-packages) were compared between DPI and MDI groups. The richness and the Shannon index were used

to assess the differences in α diversity. The Wilcoxon rank-sum test evaluated the significant difference in α diversity. The Bray-Curtis and weighted UniFrac distance measures were used to compare the two groups' β diversity with p-values calculated by PERMANOVA models after adjusting for multiple covariates (as defined in the DAG, Figure 2). A p-value compared between the two groups using the Analysis of Compositions of Microbiomes with the Bias Correction (ANCOM-BC) method by ANCOMBC R-package, [28] including an internal normalization. Taxons present in $\geq$ 5% of samples, [20,29] and covariates, as defined in the DAG (Figure 2), were included in the ANCOM-BC differential abundance model. Multiple testing was corrected using the Benjamini–Hochberg method, with 0.05 as a significant cut-off if applicable. R (version 4.2.2, 2022-10-31) and R-studio (version 2023.03.1+446) were used for the analyses and data visualizations.

Results

Children's baseline demographics and clinical characteristics are shown in Table 1 (n_{Total}=143, 41% females, n_{DPI} group=65, n_{MDI} group=77; one participant was excluded from the analysis due to the simultaneous usage of both device types). Children in the DPI group were significantly older than the MDI group (12.8±2.2 and 10.8±3, respectively; p-value<0.001). There was no significant difference in sex between the DPI and MDI groups. Regarding ethnicity, a higher proportion of children were European (Caucasian) in the DPI group compared to the MDI group (86% versus 70%). There was a significant difference between the two groups in the country of inclusion (p-value < 0.001). Children in the MDI group were more often included in Germany (42%) and only one participant was included from Slovenia. However, in the DPI group, patients were often recruited in Spain (42%), and a few patients (9%) in Germany. The two groups had no differences in asthma control status (controlled versus uncontrolled) and (childhood) asthma control test ((c)ACT [30,31]) scores. However, patients in the MDI group had more often severe asthma (steps 4 and 5 based on GINA guidelines [1]) compared to the DPI group (66% versus 40% had severe asthma). Both FEV_1 % predicted before and after salbutamol were higher in the MDI group compared to the DPI group. Children in the MDI group took higher ICS dosages and had higher daily intervals than those in the DPI group. Most of the children (93.5%) in the MDI group used a spacer while using MDI devices. There were no differences in medication adherence between the two groups, and most of the children reported being adherent to medication based on the MARS-5 questionnaire. [32] The inhaler technique score was lower in the MDI group than in the DPI group. However, 45% and 17% of the data for inhaler technique were missed (NA) for MDI and DPI groups, respectively.

Global Diversity

According to the bioinformatics pipeline, with 100% accuracy in the mock communities, bacterial taxa were identified correctly at the genus level. In total, 2677 ASVs were identified, of which Bacteroidota, Firmicutes, and Proteobacteria were the most abundant bacterial phyla, and *Prevotella* , *Alloprevotella* , and *Veillonella* were the most abundant bacterial genera in the whole study population (N=142) (Figures 3 and 4). In terms of α diversity (microbial diversity within samples), there were no differences in the number of observed unique ASV per sample (adjusted p-value= 0.476), Shannon index (adjusted p-value=0.559), or CHAO1 index (adjusted p-value=0.492). Regarding the β diversity (microbial diversity between samples), PERMANOVA models showed significant differences in both Bray-Curtis (adjusted p-value=0.018) and weighted UniFrac (adjusted p-value=0.039) distance measures.

Differential abundance

Only 450 ASVs remained, after considering the ASVs presented in ≥5% of all samples. At the family level, only *Carnobacteriaceae* (Adjusted p-value=0.033), and at the genus level, both *Granulicatella* (Adjusted p-value=0.021) and *Aggregatibacter* (Adjusted p-value=0.011) showed significantly higher abundance in the MDI group compared to the DPI group (Figure 5).

Discussion

The difference in β diversity and the distinct differential abundance of certain bacterial taxa in saliva samples from children with moderate to severe asthma who used different inhaled corticosteroid devices (MDI versus

DPI) shed light on the potential impact of these devices on the saliva microbiome. Understanding how different inhaler devices affect oral cavity microbiome composition is important, as it may have implications for personalized therapy, choosing optimal devices, and reducing adverse effects in pediatric asthma.

The lack of significant difference in α diversity between MDI and DPI users suggests that overall microbial richness and evenness within individual saliva samples were similar regardless of inhaler device types. However, the β diversity analysis (microbial diversity between samples) revealed significant differences between the MDI and DPI groups. The global diversity-related findings imply that while overall α-diversity within samples may not be affected, specific compositions and taxa of the saliva bacteriome may be associated with the inhaler device type.

Identifying specific bacterial taxa with significant differential abundance between MDI and DPI users provides further insight into the potential impact of inhaler type on saliva and oral bacterial composition. The higher relative abundance of *Carnobacteriaceae* at the family level and *Granulicatella* and *Aggregatibacter* at the genus level in the MDI group suggests that these taxa may be associated with factors unique to MDI usage, such as formulation and administration technique. *Granulicatella* and*Aggregatibacter* members are among normal oral flora bacteria that may cause serious infections and diseases like infective endocarditis and aggressive periodontitis. [33]*Aggregatibacter* genera, previously known as*Actinobacillus,* [34] is a Gram-negative bacteria from the *Pasteurellaceae* family and associated with periodontal infection, [35,36] endocarditis, and pneumonia.[37] A longitudinal study of 700 adolescents showed the association between aggressive periodontitis and *Aggregatibacter actinomycetemcomitans* in plaque samples. [38] In addition, a systematic review with meta-analysis reported a significant association between periodontal disease and asthma. [39]*Granulicatella* is from the *Carnobacteriaceae* family and is a Gram-positive lactic acid bacterium. [33]*Granulicatella* species were shown to be associated with periodontitis, [40] severe childhood caries,[41] and endodontic infections. [42,43]Understanding the role of these bacterial taxa in oral health, asthma pathogenesis, and their response to corticosteroid inhalation and deposition could provide valuable insights into disease management strategies.

ICS treatment has been reported as a significant contributor to the variability in oral microbiome compositions. Local deposition of ICS may lead to side effects like dysphonia, and oropharyngeal candidiasis, which can be prevented by rinsing the mouth and throat with water after use. [12-15] These side effects add an extra burden to the disease burden on children and their caregivers. Increased doses of ICS intake may result in greater deposition of corticosteroids in the oral cavity and may have a higher impact on oral microbiota composition. Nevertheless, we included the daily doses of ICS intake in our analyses. We have adjusted the analyses, such as global diversity (α and β diversities) and differential abundance analyses (ANCOM-BC) for this confounding factor.

Despite demonstrating lower lung function, children in the DPI group received lower ICS dosages with less frequent intervals and showed a comparable level of asthma control to the MDI group (62%). Additionally, the DPI group exhibited a higher reversibility response to salbutamol, indicating more room for improvement in their lung function. It is important to note that asthma severity should be taken into account, as fewer children in the DPI group had severe asthma (40%) compared to the MDI group (66%). Children with lower lung function may have less respiratory force to inhale and sufficiently transfer corticosteroids into their lungs. So, lower lung function may lead to higher corticosteroid deposition. Which should be taken into account for further research.

This study had multiple strengths. It is the first study to focus on assessing the association between inhaler devices and bacterial microbiome in saliva. It is a multinational European cohort, which increases the generalizability of findings compared to single-center studies. In this study, comprehensive medication intake information, including medications, dosage, intervals, and device type from the last year before inclusion time and/or at the inclusion, was collected by physicians to obtain more accurate data and report more reliable results. In addition, we could assess medication adherence and inhaler techniques and incorporate them into the analyses, which is vital in investigating the association between inhaler devices and saliva microbiome.

There are also limitations related to this study. We do not have information about oral health that can influence the oral microbiome and bacterial composition in saliva. In addition, we have not assessed fungal information from saliva samples; therefore, further research should investigate the link between the salivary fungal and bacterial composition in relation to inhaler device usage. Moreover, even though we assessed medication adherence and inhaler techniques using validated methods, more objective methods like digital inhalers are suggested for future research.

Our findings contribute to a better understanding of the association between inhaler types and oral microbiome and may help optimize personalized treatment in pediatric asthma in the future. The findings indicate that inhaler device types should be recognized as potential confounding factors in asthma microbiome studies. However, more research is needed to further validate the findings and investigate the causal relation between inhaler devices and saliva microbiome.

Conclusion

The findings of this study underscore the importance of considering inhaler type as a potential factor influencing saliva microbiome composition in children with moderate-to-severe asthma. Further research is needed to elucidate the underlying mechanisms driving these differences and to determine the clinical implications of altered microbiome composition on asthma outcomes and oral health. Understanding the impact of inhaler devices on microbiome composition and microbial dysbiosis may ultimately inform personalized asthma management strategies tailored to individual microbial profiles.

Ethical Approval

This study was carried out in accordance with the Declaration of Helsinki and approved by the local MRB of all study centers; the Medical Ethics Committee of the University Medical Center Utrecht, Utrecht, the Netherlands (NL55788.041.15); the ethics committee of University Regensburg, Germany (18–1034–101); Clinical Research Ethics Committee of the Basque Country, Spain (PI2015075 (SO)); National Medical Ethics Committee, Slovenia (0120–569/2017/4)). The SysPharmPediA study was registered at Clinicaltrials.gov with the identifier of NCT04865575. All participants/parents gave their informed consent.

Author contributions

AHAB, MIAA, SiH, SJHV, and AHM contributed to the study design, implementation, data and statistical analysis, interpretation of the results, and writing the original draft. All co-authors participated in data collection, had access to the database, provided their feedback, and approved the final version of the manuscript.

Conflict of interest

AHAB, MIAA, SiH, CB, SB, PCE, AF, SuH, PK, LLF, OSP, AAT, CW, and ADK have no conflicts of interest to disclose. MG received the SysPharmPedia grant, co-financed by the Ministry of Education, Science and Sport Slovenia (MIZS) (contract number C3330-16-500106) and funded by Slovenian Research Agency (research core funding No. P3-0427). AHN has received the ERANET Systems Medicine and ZonMW grant [project number: 9003035001], which was paid to the institution. MPY received funding from ISCIII through AES and EC within the AAL framework, and the SysPharmPedia grant from the ERACoSysMed 1st Joint Transnational Call from the European Union under the Horizon 2020 (AC15/00015), and also funded by grants SAF2017-83417R and PID2020-116274RB-I00 from MICIU/AEI/10.13039/501100011033 Spanish Ministry of Science, Innovation and Universities (outside of the submitted work), by the Ramon y Cajal program (RYC-2015-17205) by MICIU/AEI/10.13039/501100011033 Spanish Ministry of Science, Innovation and Universities. She also received grant support outside of the submitted work from GlaxoSmithKline and CSL Berhing. UP received SysPharmPedia grant, co-financed by the Ministry of Higher Education, Science and Innovation Slovenia (MVZI) (contract number C3330-16-500106), Slovenian Research and Innovation Agency (research core funding No. P3-0427 and research grant No. J3-4497), and PERMEABLE grant co-financed by the Republic of Slovenia, Ministry of Higher Education, Science and Innovation (MVZI) (contract number C3330-19-252012). MK received funding from the German Ministry of Education and

Research Council, the European Union, the German Ministry of Education and Research, and the Bavarian Ministry of Health. He received consulting fees from AstraZeneca, honoraria from EAACI, Novartis, Nutricia, and Pari. SJHV received support from SysPharmPediA ERANET and ZonMW, which was paid to the institution. AHM is the PI of a public-private consortium (P4O2 (Precision Medicine for More Oxygen)) sponsored by Health Holland involving many private partners that contribute in cash and/or in kind (AbbVie, Boehringer Ingelheim, Breathomix, Fluidda, Ortec Logiqcare, PeXA, Philips, Quantib-U, Smartfish, Clear, SODAQ, Thirona, Roche, TopMD, Novartis, RespiQ). She received unrestricted research grants from GSK, Boehringer Ingelheim and Vertex, and honoraria from Boehringer Ingelheim, AstraZeneca and GSK. She is the chair of a DSMB of a study on BPD in neonates.

Support statement

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Acknowledgment

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Data availability

For clinical and other data generated within the SysPharmPediA study, the authors will make them available upon specific requests subject to the requestor obtaining ethical, research, data access, and collaboration approvals from the SysPharmPediA study management board. Requests can be sent to a.h.maitland@amsterdamumc.nl.

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Figure 1. Descriptive study flow chart. Participants were categorized based on the type of inhaled corticosteroid device: DPI: dry powder inhalers; MDI: metered dose inhalers.

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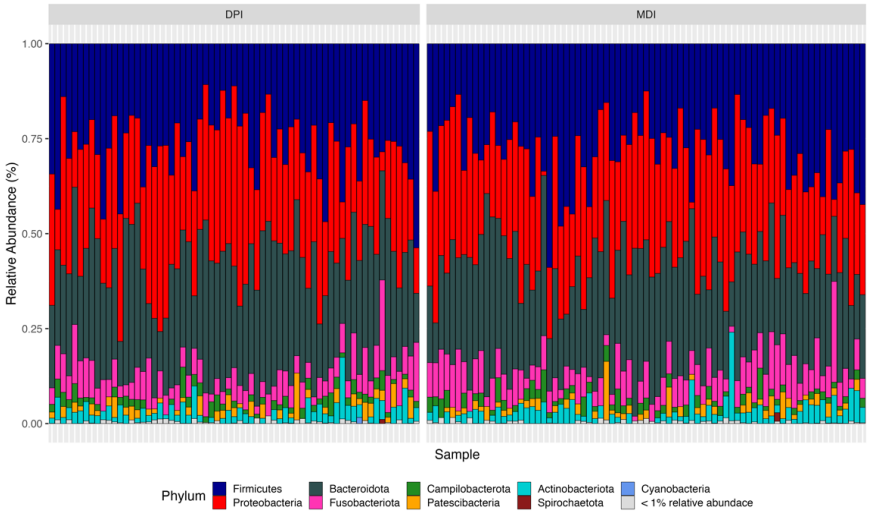
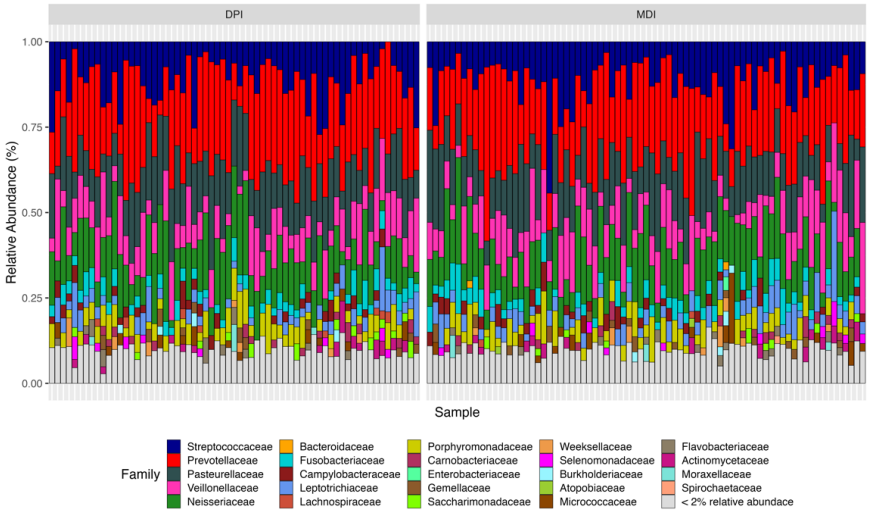
Figure 2. DAG (Directed Acyclic Graph). Created by www.daggitty.net. ICS: inhaled corticosteroid.





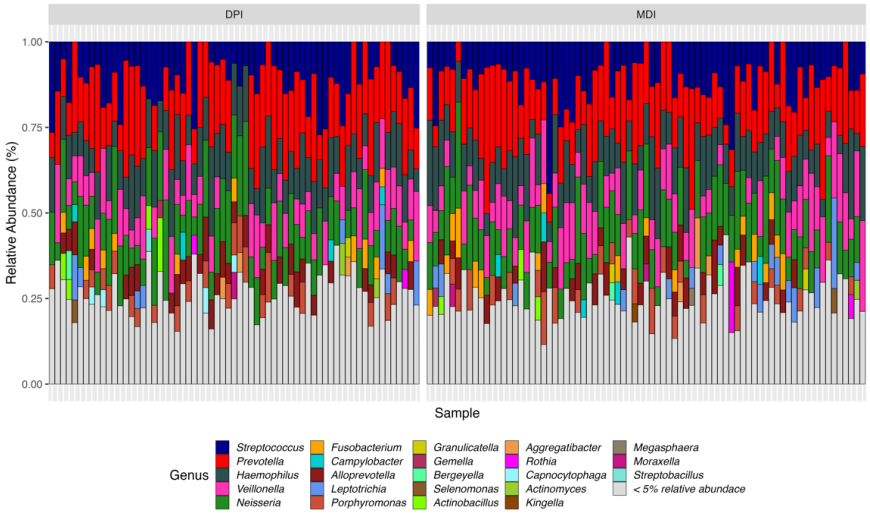
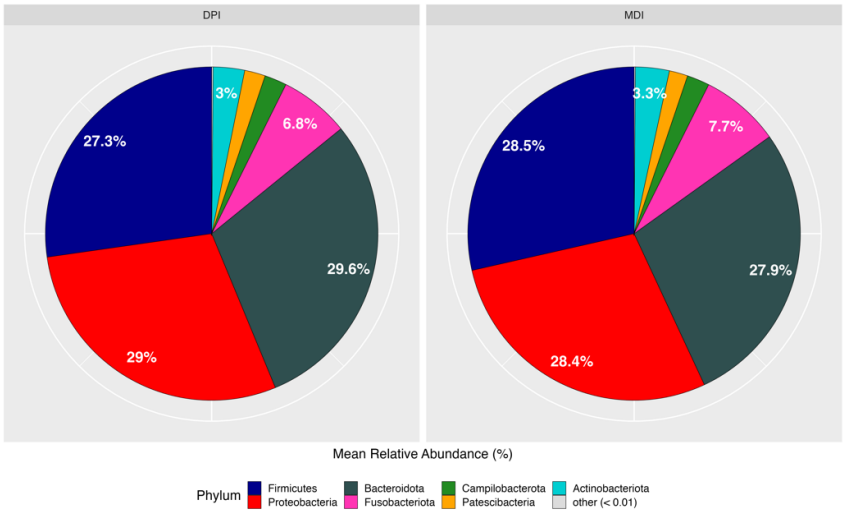


Figure 3. Relative abundance of samples at the Phylum (A), Family (B), and Genus (C) levels between the children using DPI and children using MDI. DPI: dry powder inhalers; MDI: metered dose inhalers.



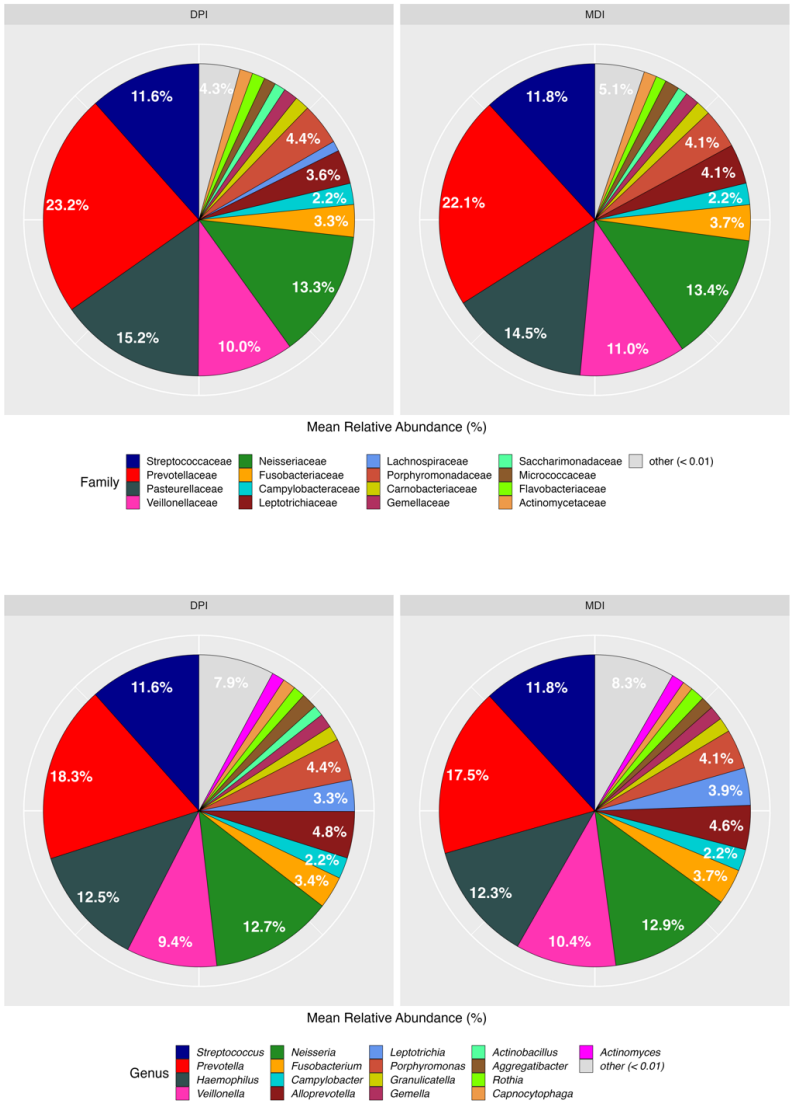


Figure 4. Mean relative abundance of samples at the Phylum (A), Family (B), and Genus (C) levels between the children using DPI and children using MDI. DPI: dry powder inhalers; MDI: metered dose inhalers.

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Figure 5. Bacterial taxa with significant differential abundance between children using DPI (in red) and children using MDI (in blue). DPI: dry powder inhalers; MDI: metered dose inhalers.

Table 1. Demographic and clinical characteristics of the participants in DPI and MDI groups.

Characteristics	**DPI (n=65)**	**MDI (n**
Demographics		

Characteristics	**DPI (n=65)**	**MDI (n**
Age, years, mean (SD)	12.8 (2.2)	10.8 (3.0)
Female, n (%)	28/65 (43%)	30/77 (39
Ethnicity, n (%)		
Caucasian	56/65 (86%)	54/77 (70
Non-Caucasian	9/65 (14%)	23/77 (30
Body mass index (BMI) z-score, mean (SD)	0.47 (1.37)	0.57 (1.2
Smoking exposure, n (%)	22/61 (36%)	18/74 (24
Country of inclusion, n (%)		
Spain	27/65 (42%)	23/77 (30
Germany	6/65 (9%)	32/77 (42
The Netherlands	10/65 (15%)	21/77 (27
Slovenia	22/65 (34%)	1/77 (1%
Clinical characteristics		
Asthma control status, n (%)		
Uncontrolled	40/65 (62%)	48/77 (62
Asthma Severity, n (%)		
Moderate	39/65 (60%)	26/77 (34
Severe	26/65 (40%)	51/77 (66
ACT score, median (IQR)	23 (20, 25) (n=65)	22 (18, 24
Lung function test, median (IQR)		
FEV_1 % predicted pre-salbutamol	90.7 (81.1, 98.2) (n=64)	97.5 (85.8
FEV_1 % predicted post-salbutamol	98.3 (89.0, 103.2) (n=64)	102.6 (92
Bronchodilator reversibility (Change in FEV_1[?]12% after salbutamol intake), n (%)	21/64 (33%)	11/73 (15
ICS type, n (%)		
Beclomethasone	4/65 (6%)	11/77 (14
Budesonide	14/65 (22%)	0/77 (0%
Ciclesonide	0/65 (0%)	1/77 (1%
Fluticasone	47/65 (72%)	65/77 (84
ICS dosage*, n (%)		
Low	40/65 (62%)	26/77 (34
Medium	10/65 (15%)	36/77 (47
High	15/65 (23%)	15/77 (19
ICS intervals (per day), n (%)		
1	15/65 (23%)	1/77 (1%
2	45/65 (69%)	34/77 (44
3	1/65 (2%)	0/77 (0%
4	3/65 (5%)	40/77 (52
6	1/65 (2%)	2//77 (3%
Spacer used with ICS device, n (%)	—	72/77 (93
Medication Adherence based on MARS-5 questionnaire, n (%)		
Nonadherent (score <23)	14/61 (23%)	15/67 (22
Adherent (score ≥23)	47/61 (77%)	52/67 (78
Inhaler Technique Score, median (IQR)	100 (100, 100) (n=54)	91 (91, 10

FEV_1: Forced Expiratory Volume in One Second; ICS: Inhaled CorticoSteroids.

***** Based on GINA guideline 2016, Box 8, page 15.

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