**SUPPLEMENTARY MATERIALS**

# Antibiotics in pregnancy influence nasal microbiome and respiratory morbidity in infancy

Céline Rüttimann[1,2], Annika Nissen-Kratzert[1,2], Nadja Mostacci[3], Noëmi Künstle[1,2], Andrea Marten[1,2], Amanda Gisler[1,2], Katharina Bacher[3], Sophie Yammine[1,2], Ruth Steinberg[1,2,4], Sven Schulzke[1,2], Martin Röösli[5], Philipp Latzin[1,2], Markus Hilty[3], Urs Frey[1,2*], and Olga Gorlanova[1,2*] on behalf of the BILD study group

[1] University Children's Hospital Basel (UKBB), University of Basel, Basel, Switzerland

[2] Division of Paediatric Respiratory Medicine and Allergology, Department of Paediatrics, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

[3] Institute for Infectious Diseases, University of Bern, Bern, Switzerland

[4] Graduate School for Cellular and Biomedical Sciences, University of Bern, Switzerland

[5] Swiss Tropical and Public Health Institute, Allschwil, Switzerland and University of Basel, Basel, Switzerland

*shared last authorship

**Corresponding author:**

Prof. Urs Frey, University Children's Hospital Basel (UKBB), Switzerland

Spitalstrasse 33, 4056 Basel, Switzerland, email: urs.frey@ukbb.ch

**CONTENTS**

**Supplementary methods**

Study design and study population

The Basel-Bern Infant Lung Development (BILD) study, a prospective birth cohort study ongoing since 1999, aims to investigate respiratory diseases (especially asthma) in childhood and their causes. Research focus was placed primarily on influence of environmental factors (indoor and outdoor air pollution), viral infections and genetic predisposing factors on lung development, also with emphasis on at-risk children. Participants, respectively their parents, are recruited prenatally or at the latest three weeks after birth at two Swiss university hospitals (Department of Gyneacology and Obstetrics at Inselspital, Bern University Hospital and University Hospital of Basel). More details about the Basel-Bern Infant Lung Development (BILD) cohort study and the entire study process are described elsewhere [1]. In total, more than 1000 children have already been included in the BILD cohort study.

For this study, a subset of the BILD study consisting of term children with nasal swabs at age 4–6 weeks, no postnatal antibiotic exposure before swab and the complete follow-up for respiratory symptoms was analysed. For further information about inclusion and exclusion criteria, please see study population and study sample in the main manuscript.

Data Collection

Anterior nasal swabs were collected at an appointment at the study clinic at age 4–6 weeks. For the present study, children with swabs between April 2010 and July 2020 were included. Trained study nurses used 2 flexible, sterile swabs (FLOQSwabs® 516CS01, Copan; Italy) to collect anterior nasal swabs from both nostrils. The swabs were inserted into the nostrils until the entire padded part was in contact with the nasal mucosa, and were then brushed along the mucosa several times in circular movements. Both swabs were placed together in a tube with 3ml stabilizing medium (UTM-RTTM in Screw-Cap Tube, Copan; Italy) and tubes were stored at room temperature for 2–5 hours. The solution, containing medium and nasal secretion, was shaken in the tubes, divided into 3 aliquots of

1ml each using an Eppendorf pipette and then placed into micro-screw tubes (Sarstedt; Nürnbrecht, Germany). Afterwards, the tubes were frozen and kept at -80°C until further processing.

All clinical and personal data about pregnancy and birth was taken from medical reports or raised during the interview with the parents at the age 4-6 weeks visit. This included important factors such as sex, gestational age, birth mode, maternal atopy, maternal smoking in pregnancy, type of infection in third trimester. Clinical information about health and especially respiratory infections of the child and important factors in the first year of life (duration of breastfeeding, presence of siblings, attendance in childcare) were collected during the weekly telephone interviews by trained study nurses.

Bacterial DNA extraction

After isolation of bacterial DNA, amplification of the variable regions (V3 to V4) of the bacterial 16S-rRNA gene and next generation sequencing, the phylogenetic library was constructed.

Due to excessive dilution with medium, bacterial density was too low in a majority of the samples for the first attempts of 16S-rRNA gene amplification. For the purpose of obtaining a higher concentration of bacterial DNA, the initial solution was centrifuged with 12000g for 5 minutes and supernatant was discarded, resulting in a residual amount of 200µl. With this change in procedure, the test samples showed a significantly higher DNA concentration after 16S rRNA amplification.

All other frozen samples were sent to the external company Eurofins Genomics (Ebersberg, Germany) for complete processing after the successful first round. After all samples had thawed, they were centrifuged with 12000g for 5 minutes and the supernatant was discarded except for the required 200µl. The subsequent steps of DNA extraction were performed using the NucleoSpin Food Kit (Macherey-Nagel; Düren, Germany). The pellets including 200µl supernatant were mixed with 200µl LysisBuffer CF (preheated to 65°C) and pre-texted. Then, another 350µl of LysisBuffer CF (preheated to 65°C) was mixed in and 10µl of Proteinase K was added. Before further processing according to the Macherey-Nagel protocol, the samples were incubated overnight at 65°C.

16S-rRNA Amplification and Next Generation Sequencing

The extracted DNA was further processed by 16S-rRNA amplification. The primer pair 357F (TACGGGAGGCAGCAG)/800R (CCAGGGTATCTAATCC) was used for the amplification of the hypervariable V3 and V4 regions of the bacterial 16S-rRNA genes in the present study and each sample was passed through 25 PCR cycles. More detailed descriptions of 16S-rRNA amplification can be found elsewhere [2].

The MiSeq [300PE] Platform (Illumina; San Diego, CA, USA) was used for next generation sequencing (sequencing-by-synthesis). Equimolar amplicon pools were sequenced in 3 runs, with an estimated data-output of 112,000 readpairs per sample. Internal company protocols and manufacturer's specifications were used for 16S-rRNA amplification and next generation sequencing.

Quality control

Nasal swabs in newborns show low bacterial density [3], therefore different quality controls were performed to control for possible contamination. Negative extraction controls were analysed along with the samples: each showed no sequenceable material and thus no contamination. Since possible influence of contamination is greater with lower bacterial density of the sample, samples with DNA concentration less than 0.2ng/µl after PCR were excluded for microbiome analysis (in present analysis this equated to 34 samples with a PCR product concentration below 0.2ng/µl after 25 PCR cycles). Due to these findings no co-clustering with DNA blanks was possible or necessary.

4 samples were taken by the parents at home and therefore had to be excluded due to lack of quality standards. One sample was excluded due to a viral infection at date of nasal swab.

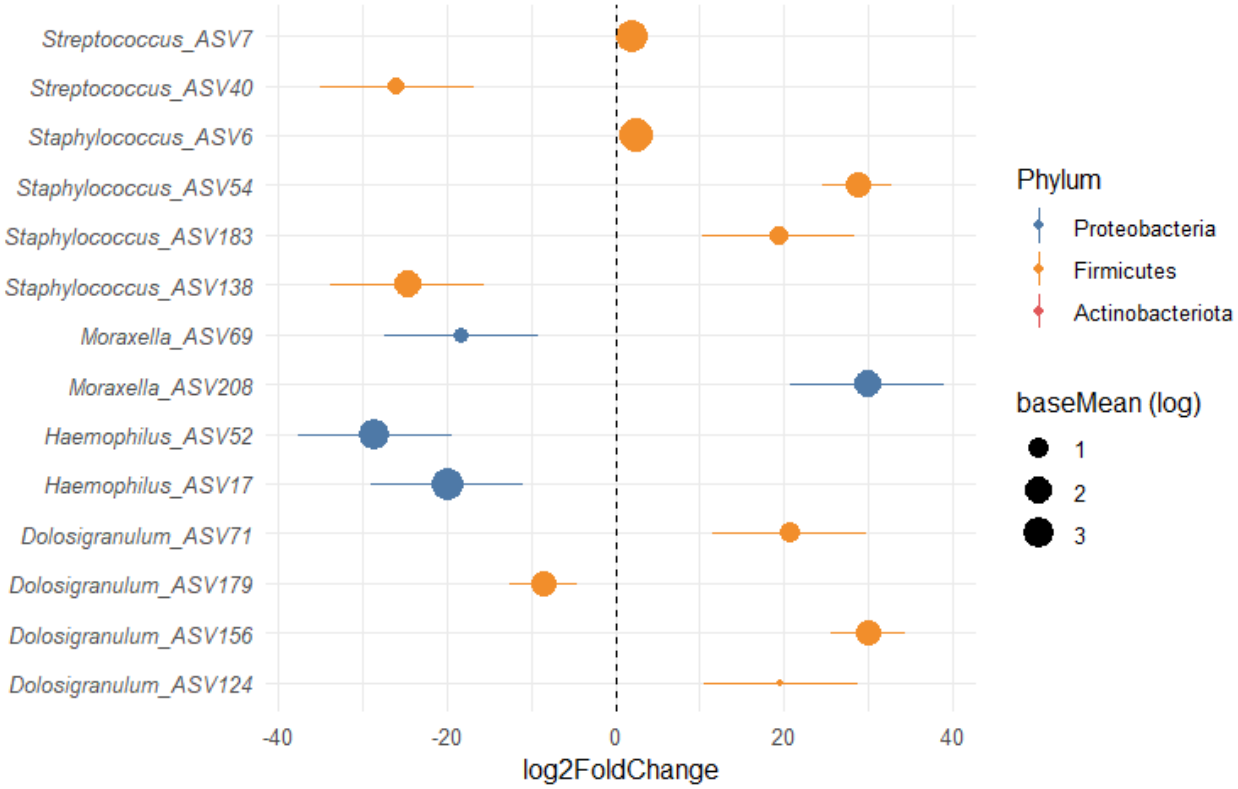
Microbial processing

The raw sequencing reads were processed with DADA2 (version 1.18.0) [4]. The forward and reverse reads were trimmed to the length of 280 bp and 200 bp respectively, otherwise default parameters were used. Silva database version 138.1 [5] was used for assigning taxonomy. The 16S amplicon data are available under project PRJNA944094. All microbiome analyses were done in R (Version 4.2.0) [6] using the *phyloseq* package [7].

**Supplementary references**


1. Fuchs O, Latzin P, Kuehni CE, *et al.* Cohort profile: the Bern infant lung development cohort. *International journal of epidemiology* 2012: 41(2): 366-376.
2. Hilty M, Qi W, Brugger SD, *et al.* Nasopharyngeal microbiota in infants with acute otitis media. *The Journal of infectious diseases* 2012: 205(7): 1048-1055.
3. Biesbroek G, Sanders EA, Roeselers G, *et al.* Deep sequencing analyses of low density microbial communities: working at the boundary of accurate microbiota detection. *PloS one* 2012: 7(3): e32942.
4. Callahan BJ, McMurdie PJ, Rosen MJ, *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nature methods* 2016: 13(7): 581-583.
5. Yarza P, Yilmaz P, Pruesse E, *et al.* Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature reviews Microbiology* 2014: 12(9): 635-645.
6. The R Foundation. The R Project for Statistical Computing. 2023 [cited 2023 Feb. 6]; Available from: https://www.r-project.org/
7. McMurdie PJ, Holmes S. Phyloseq: a bioconductor package for handling and analysis of high-throughput phylogenetic sequence data. *Pacific Symposium on Biocomputing Pacific Symposium on Biocomputing* 2012: 235-246.

**Supplementary Figure S1:** Differential abundance analysis, species level




ASV7: Streptococcus mitis group; ASV40: Streptococcus pneumoniae; ASV6/54: Staphylococcus epidermidis; ASV138/183: Staphylococcus aureus; ASV69/208: Moraxella lincolnii; ASV17/52: Haemophilus influenzae;
ASV17//71/124/156/179: Dolosigranulum pigrum
Positive log2FoldChange indicates a lower abundance in infants with exposure to antibiotics in 3rd trimester.

**Supplementary Table S1:** Summary statistics for follow-up at age 6 years

| | Total n=158 | No antibiotics in 3rd trimester n=137 | Antibiotics in 3rd trimester n=21 | p-value[a] |
|---|---|---|---|---|
| Sex, male | 72 (45.6%) | 61 (44.5%) | 11 (52.4%) | 0.501 |
| Gestational age, weeks | 39.8 (1.1) | 39.8 (1.1) | 39.9 (0.7) | 0.611 |
| Duration of any breastfeeding, weeks | 32.2 (13.8) | 31.8 (13.5) | 35.0 (15.5) | 0.335 |
| Birth mode, Caesarean section | 41 (25.9%) | 35 (25.5%) | 6 (28.6%) | 0.768 |
| Presence of older siblings | 101 (63.9%) | 88 (64.2%) | 13 (61.9%) | 0.836 |
| Attendance in childcare | 70 (44.3%) | 60 (43.8%) | 10 (47.6%) | 0.743 |
| Study centre, Basel | 73 (46.2%) | 60 (43.8%) | 13 (61.9%) | 0.121 |
| Maternal atopy[b] | 63 (39.9%) | 52 (38.0%) | 11 (52.4%) | 0.209 |
| Intrapartum antibiotic prophylaxis (IAP) | 26 (16.5%) | 25 (18.2%) | 1 (4.8%) | 0.121 |
| Maternal infections in third trimester requiring antibiotic treatment | | | | |
| Urinary tract/gynaecological infection | 9 (5.7%) | - | 9 (42.9%) | |
| Respiratory infection | 4 (2.5%) | - | 4 (19.0%) | |
| ENT infection[c] | 4 (2.5%) | - | 4 (19.0%) | |
| Other infection[c] | 1 (0.6%) | - | 1 (4.8%) | |
| Unknown | 3 (1.9%) | - | 3 (14.3%) | |
| Wheeze between 2–6 years, yes | 41 (25.9%) | 35 (25.5%) | 6 (28.6%) | 0.768 |
| Atopy child with 6 years, yes | 29 (18.4%) | 25 (18.2%) | 4 (19.0%) | 0.930 |

Data is presented as mean (SD) for continuous and count measures, n (%) for categorical measures.
[a] p-values were obtained using t-test, Mann-Whitney test, Pearson $\chi^2$ and Fisher exact test, as appropriate
[b] defined as bronchial asthma, allergic rhinitis or atopic dermatitis
[c] defined as infection of ears, neck, throat, e.g. otitis media
[d] defined as infection not classified above, e.g. gastrointestinal infection

**Supplementary Table S2:** Effect of antibiotics in 3rd trimester on any/severe respiratory symptoms in 1st year of life (direct and indirect effect) with other microbiome measurements as mediators

| | | | **Any respiratory symptoms** | | | **Severe respiratory symptoms** | | |
|---|---|---|---|---|---|---|---|---|
| | | | **β/RR[a]** | **95% CI[b]** | **p-value ($p_{adj}$-value)** | **β/RR[a]** | **95% CI[b]** | **p-value ($p_{adj}$-value)** |
| α-diversity | Shannon | direct | 1.38 | 1.03;1.84 | **0.031 (0.032)** | 1.75 | 1.02;2.97 | **0.041 (0.041)** |
| | | indirect | -0.02 | -0.06;0.03 | 0.461 (0.527) | 0.01 | -0.08;0.10 | 0.808 (0.903) |
| | | total | 0.30 | 0.01;0.59 | **0.041 (0.041)** | 0.57 | 0.04;1.10 | **0.037 (0.038)** |
| | Simpson | direct | 1.38 | 1.03;1.84 | **0.032 (0.032)** | 1.75 | 1.02;2.98 | **0.040 (0.041)** |
| | | indirect | -0.01 | -0.06;0.03 | 0.553 (0.527) | 0.01 | -0.08;0.10 | 0.908 (0.903) |
| | | total | 0.31 | 0.02;0.60 | **0.039 (0.041)** | 0.56 | 0.03;1.09 | **0.038 (0.038)** |
| β- diversity | Pcoa1 | direct | 1.36 | 1.02;1.82 | **0.038 (0.046)** | 1.79 | 1.05;3.06 | **0.032** (0.064) |
| | | indirect | 0.01 | -0.01;0.03 | 0.569 (0.563) | -0.01 | -0.05;0.03 | 0.588 (0.570) |
| | | total | 0.31 | 0.02;0.60 | **0.035 (0.035)** | 0.57 | 0.04;1.11 | **0.035 (0.045)** |
| | Pcoa2 | direct | 1.35 | 1.01;1.79 | **0.046 (0.046)** | 1.55 | 0.92;2.63 | 0.098 (0.098) |
| | | indirect | 0.03 | -0.01;0.08 | 0.174 (0.362) | 0.10 | -0.02;0.21 | 0.093 (0.214) |
| | | total | 0.33 | 0.04;0.62 | **0.028 (0.035)** | 0.54 | 0.01;1.07 | **0.045 (0.045)** |
| Abundance | *Staphylococcus* | direct | 1.36 | 1.02;1.82 | **0.037 (0.043)** | 1.75 | 1.03;2.98 | **0.038** (0.058) |
| | | indirect | $-6.16 \times 10^{-8}$ | $-9.88 \times 10^{-7}$; $1.11 \times 10^{-6}$ | 0.908 | $1.01 \times 10^{-7}$ | $-1.63 \times 10^{-6}$; $1.83 \times 10^{-6}$ | 0.909 |
| | | total | 0.31 | 0.02; 0.60 | **0.037 (0.043)** | 0.56 | 0.03;1.09 | **0.038** (0.058) |
| | *Streptococcus* | direct | 1.36 | 1.02;1.82 | **0.037 (0.043)** | 1.78 | 1.05;3.04 | **0.033** (0.058) |
| | | indirect | $3.73 \times 10^{-8}$ | $-2.14 \times 10^{-6}$; $2.21 \times 10^{-6}$ | 0.973 | $3.14 \times 10^{-6}$ | $-5.61 \times 10^{-6}$; $1.19 \times 10^{-5}$ | 0.482 |
| | | total | 0.31 | 0.02;0.60 | **0.037 (0.039)** | 0.58 | 0.05;1.11 | **0.033** (0.058) |
| | *Moraxella* | direct | 1.35 | 1.02;1.82 | **0.039 (0.043)** | 1.72 | 1.01;2.93 | **0.046** (0.058) |
| | | indirect | $2.76 \times 10^{-7}$ | $-1.22 \times 10^{-6}$; $1.77 \times 10^{-6}$ | 0.718 | $1.25 \times 10^{-6}$ | $-2.87 \times 10^{-6}$; $5.36 \times 10^{-6}$ | 0.553 |
| | | total | 0.31 | 0.02;0.60 | **0.039 (0.043)** | 0.54 | 0.01;1.07 | **0.046** (0.058) |
| | *Dolosigranulum* | direct | 1.36 | 1.02;1.82 | **0.039 (0.043)** | 1.73 | 1.02;12.94 | **0.043** (0.058) |
| | | indirect | $2.29 \times 10^{-7}$ | $-1.31 \times 10^{-6}$; $1.77 \times 10^{-6}$ | 0.770 | $4.63 \times 10^{-7}$ | $-2.80 \times 10^{-6}$; $3.72 \times 10^{-6}$ | 0.781 |
| | | total | 0.31 | 0.02;0.60 | **0.039 (0.043)** | 0.55 | 0.02;1.08 | **0.043** (0.058) |
| | *Corynebacterium* | direct | 1.34 | 1.01;1.80 | **0.043 (0.043)** | 1.67 | 0.98; 2.85 | 0.058 (0.058) |
| | | indirect | $4.14 \times 10^{-6}$ | $-1.37 \times 10^{-6}$, $9.66 \times 10^{-6}$ | 0.141 | $7.66 \times 10^{-6}$ | $-5.16 \times 10^{-6}$; $2.05 \times 10^{-5}$ | 0.242 |
| | | total | 0.30 | 0.01;0.59 | **0.043 (0.043)** | 0.52 | -0.02;1.05 | 0.058 (0.058) |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval*

Estimates for any respiratory symptoms and severe respiratory symptoms were obtained using negative binominal regression and structural equation modelling with adjustment for sex, birth mode, weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, and study centre.

[a] RR was calculated by exponentiation for direct effect after using negative binominal regression. No RR can be calculated for indirect and total effect, because of included linear regression in the model.

[b] CI is displayed exponentiated for direct effect.

[c] $p_{adj}$-value was obtained using Benjamini-Hochberg correction for multiple comparisons within group (e.g., direct effect α-diversity measures).

**Supplementary Table S3** Effect of antibiotics in 3rd trimester on secondary outcomes (wheeze between 2-6 years and atopy at 6 years)

| | | **Wheeze between 2-6 years** | | | **Atopy at 6 years** | | |
|---|---|---|---|---|---|---|---|
| | | β /OR[a] | 95% CI[b] | p-value | β /OR[a] | 95% CI[b] | p-value |
| Shannon | direct | 1.00 | 0.32;3.19 | 0.994 | 1.46 | 0.41;5.27 | 0.560 |
| | indirect | 0.06 | -0.20;0.32 | 0.650 | -0.09 | -0.37;0.19 | 0.546 |
| | total | 0.07 | -1.06;1.19 | 0.910 | 0.29 | -0.95;1.54 | 0.643 |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval*

Estimates for wheeze between 2–6 years and atopy at 6 years were obtained using logistic regression and structural equation modelling with adjustment for sex, birth mode weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, and study centre.

[a] OR was calculated by exponentiation for direct effect after using logistic regression. No OR can be calculated for indirect and total effect, because of included linear regression in the model.

[b] 95% CI is displayed exponentiated for direct effect.

**Supplementary Table S4:** Sensitivity analysis: special adjustment for IAP

| | | Any respiratory symptoms | | | Severe respiratory symptoms | | |
|---|---|---|---|---|---|---|---|
| | | β/RR[a] | 95% CI[b] | p-value | β/RR[a] | 95% CI[b] | p-value |
| Shannon | direct | 1.38 | 1.03;1.84 | **0.032** | 1.75 | 1.03/3.00 | **0.037** |
| | indirect | -0.02 | -0.06;0.03 | 0.439 | 0.01 | -0.07;0.10 | 0.779 |
| | total | 0.30 | 0.01;0.59 | **0.041** | 0.58 | 0.05;1.11 | **0.034** |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval; IAP: intrapartum antibiotic prophylaxis*
Estimates for any respiratory symptoms and severe respiratory symptoms were obtained using negative binominal regression and structural equation modelling with adjustment for sex, birth mode, weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, study centre, and IAP.
[a] RR was calculated by exponentiation for direct effect after using logistic regression. No RR can be calculated for indirect and total effect, because of included linear regression in the model.
[b] CI is displayed exponentiated for direct effect.

**Supplementary Table S5:** Sensitivity analysis: subgroup with exclusion of children who were only exposed to IAP (n=228)

| | | Any respiratory symptoms | | | Severe respiratory symptoms | | |
|---|---|---|---|---|---|---|---|
| | | β/RR[a] | 95% CI[b] | p-value | β/RR[a] | 95% CI[b] | p-value |
| Shannon | direct | 1.32 | 0.98;1.79 | 0.067 | 1.77 | 1.03;3.01 | **0.039** |
| | indirect | -0.01 | -0.05;0.04 | 0.370 | 0.02 | -0.08;0.11 | 0.729 |
| | total | 0.28 | -0.02;0.58 | 0.072 | 0.58 | 0.04;1.12 | **0.034** |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval; IAP: intrapartum antibiotic prophylaxis*
Estimates for any respiratory symptoms and severe respiratory symptoms were obtained using negative binominal regression and structural equation modelling with adjustment for sex, birth mode, weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, and study centre.
[a] RR was calculated by exponentiation for direct effect after using logistic regression. No RR can be calculated for indirect and total effect, because of included linear regression in the model.
[b] CI is displayed exponentiated for direct effect.

**Supplementary Table S6:** Sensitivity analysis: subgroup of children without an atopic mother (n=205)

| | | Any respiratory symptoms | | | Severe respiratory symptoms | | |
|---|---|---|---|---|---|---|---|
| | | β/RR[a] | 95% CI[b] | p-value | β/RR[a] | 95% CI[b] | p-value |
| Shannon | direct | 1.47 | 1.07; 2.03 | **0.019** | 1.86 | 1.07;3.21 | **0.027** |
| | indirect | -0.02 | -0.06;0.03 | 0.432 | -0.01 | -0.06;0.04 | 0.596 |
| | total | 0.37 | 0.04;0.70 | **0.026** | 0.61 | 0.06;1.16 | **0.031** |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval*
Estimates for any respiratory symptoms and severe respiratory symptoms were obtained using negative binominal regression and structural equation modelling with adjustment for sex, birth mode, weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, and study centre.
[a] RR was calculated by exponentiation for direct effect after using logistic regression. No RR can be calculated for indirect and total effect, because of included linear regression in the model.
[b] CI is displayed exponentiated for direct effect.

**Supplementary Table S7:** Effect of microbiome on any/severe respiratory symptoms in 1st year

| | | Any respiratory symptoms | | | Severe respiratory symptoms | | |
|---|---|---|---|---|---|---|---|
| | | **RR[a]** | **95% CI[b]** | **p-value ($p_{adj}$-value)** | **RR[a]** | **95% CI[b]** | **p-value ($p_{adj}$-value)** |
| α- | Shannon | 1.06 | 0.91;1.24 | 0.441 (0.542) | 0.96 | 0.69;1.34 | 0.808 (0.908) |
| | Simpson | 1.13 | 0.76;1.70 | 0.542 (0.542) | 0.95 | 0.41;2.20 | 0.908 (0.908) |
| β- | Pcoa1 | 0.87 | 0.60;1.25 | 0.447 (0.447) | 1.31 | 0.61;2.81 | 0.485 (0.485) |
| | Pcoa2 | 0.66 | 0.43;1.03 | 0.069 (0.138) | 0.28 | 0.12; 0.66 | **0.003 (0.006)** |
| Abundance | *Staphylococcus* | 1.00 | 0.99;1.00 | 0.306 (0.728) | 1.00 | 0.99;1.00 | 0.432 (0.540) |
| | *Streptococcus* | 1.00 | 0.99;1.00 | 0.991 (0.991) | 1.00 | 0.99;1.00 | 0.385 (0.540) |
| | *Moraxella* | 1.00 | 0.99;1.00 | 0.685 (0.856) | 1.00 | 0.99;1.00 | 0.368 (0.540) |
| | *Dolosigranulum* | 1.00 | 0.99;1.00 | 0.437 (0.728) | 1.00 | 0.99;1.00 | 0.558 (0.558) |
| | *Corynebacterium* | 1.00 | 0.99;1.00 | 0.068 (0.340) | 1.00 | 0.99;1.00 | 0.185 (0.540) |

*Abbreviations: β: regression coefficient; RR: risk ratio, CI: confidence interval*

Estimates for any respiratory symptoms and severe respiratory symptoms were obtained using negative binominal regression with adjustment for sex, birth mode, weeks of any breastfeeding, season of swab collection, maternal atopy, maternal smoking during pregnancy, presence of older siblings, childcare, and study centre.

[a] RR was calculated by exponentiation for direct effect after using logistic regression. No RR can be calculated for indirect and total effect, because of included linear regression in the model.

[b] CI is displayed exponentiated for direct effect.