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Differences of the respiratory microbiota between children suffering from community acquired pneumonia with presence or absence of asthma

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Recent advancements in respiratory microbiota research have progressively elucidated their pivotal role in pediatric respiratory pathologies. Alterations in airway microbial communities are intricately associated with diverse respiratory conditions and distinct disease states. This study conducted a comparative analysis of respiratory microecological profiles in pediatric cohorts diagnosed with community-acquired pneumonia (CAP), stratified by the presence or absence of comorbid bronchial asthma, from whom nasopharyngeal aspirates were obtained for metagenomic next-generation sequencing (mNGS). Analyses revealed comparable alpha-diversity indices between groups; however, beta-diversity metrics demonstrated marked compositional divergence. In the asthma-CAP cohort, *Streptococcus pneumoniae* and *Rothia mucilaginosa* emerged as predominant taxa, whereas *Mycoplasmoides pneumoniae* and *Trichoderma citrinoviride* dominated microbial profiles in uncomplicated CAP patients.

Keywords Asthma, Community-acquired pneumonia, Respiratory microbiota, Pathogen, Metagenomic second-generation sequencing

Community-acquired pneumonia (CAP) is the leading cause of mortality in children under five years old globally and remains one of the most prevalent infectious diseases in this age group[1,2]. The annual global incidence of CAP is close to 0.22 episodes per child per year or 155 million new cases per year in the world, of which 10–17% require hospitalization[3]. The etiology of pneumonia in the pediatric population is variable and changes according to age and disease severity and where the study is conducted[4].

Bronchial asthma (asthma) defined as a heterogeneous disease, is characterized by chronic airway inflammation and variable expiratory airflow limitation. Over 300 million people worldwide suffer from asthma, contributing to high substantial social costs[5]. The airway epithelial cells of asthma patients are damaged, with cytokines and inflammatory mediators related to allergic inflammation inducing the destruction of the epithelial barrier, thus increasing their susceptibility to infection[6]. Asthma exacerbations are a frequent cause of emergency visits in pediatric patients, with infections being a primary trigger and often leading to secondary pneumonia[7]. Anti-asthma and early anti-infection treatments are essential for improving prognosis. However, there is a scarcity of guidelines on anti-infective drugs selection for pneumonia patients with concurrent asthma.

Metagenomic next-generation sequencing (mNGS) has gained traction in the clinical diagnosis of various infectious diseases[8,9]. This unbiased detection method can rapidly identify nearly all pathogens in clinical samples, boasting high sensitivity and accuracy, particularly in diagnosing novel, rare, unknown, and atypical pathogens in complex infectious diseases[10]. The ability to detect low amounts of DNA further enhances the utility of mNGS in clinical pathogen diagnosis. Previous studies have shown that culture, the gold standard for bacteria and fungi detection[11]is susceptible to interference from antibiotic treatment[12]. Given that empirical anti-infection treatment is often initiated upon hospital admission for pneumonia, mNGS was employed in this study to comprehensively cover all pathogens and mitigate the impact of prior antibiotic use.

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We hypothesize that there are differences in airway dominant pathogens and microbiota between children with and without bronchial asthma who suffer from community - acquired pneumonia. To test this hypothesis, our study enrolled children with asthma complicated by pneumonia and children with uncomplicated CAP as subjects. We analyzed the results of metagenomic second-generation sequencing of their nasopharyngeal aspirates to explore the airway microecological characteristics in both groups. Additionally, we compared the differences in dominant pathogens between the two groups, aiming to provide reference for clinical empirical medication. Furthermore, we conducted spearman correlation analysis to explore the correlation between different microbial species obtained through sequencing and relevant clinical data, thereby further evaluating the relationship between microbial groups and the disease state of the host.

Results

Cohort characteristics of participants

In the Propensity Score Matching (PSM), covariates such as the patient's age, sex, and fever duration before sampling were taken into account. No statistically significant differences ($p > 0.05$) were noted, which confirmed the comparability between the groups. Upon comparing clinical manifestations and laboratory indicators, it was shown that in comparison to the group of children with pneumonia accompanied by asthma, those in the simple community-acquired pneumonia (CAP) group had notably higher erythrocyte sedimentation rates, D-dimer levels, and requirements for oxygen support. (Table 1).

Distinct airway microecological community structures in asthma-CAP and simple-CAP

In this investigation, no statistically significant disparities in alpha diversity were detected between the Asthma-CAP and Simple-CAP cohorts, which validated comparable species diversity and richness across the two groups (Fig. 1).

Beta diversity assessment via the Bray-Curtis distance metric demonstrated that principal coordinates analysis (PCoA) revealed the first principal coordinate accounted for 29.3% of total variance, while the second principal coordinate explained 12.9%, cumulatively accounting for 42.2% of the observed variation. This finding indicated a marked discrepancy in community diversity between groups ($P = 0.009$). Similarly, principal component analysis (PCA) showed the first principal component explained 9.8% of variance and the second component 6.8%, together contributing 16.6% of total variation, further confirming statistically significant differences in airway microecological community structure ($P = 0.014$). Non - metric multidimensional scaling (NMDS) directly visualized data trends, with results demonstrating significant differences in airway microecological communities between groups ($P = 0.019$) (Fig. 1).

Distinct phylum-level microbial profiles in asthma-CAP and simple-CAP groups

In both study cohorts, consistent phylum-level taxonomic distributions were observed. The eleven most predominant microbial phyla identified were *Bacillota*, *Mycoplasmatota*, *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, *Ascomycota*, *Fusobacteriota*, *Preplasmiviricota*, *Campylobacterota*, *Basidiomycota* and *Spirochaetota* (Fig. 2). Within the Asthma-CAP airway microbial community, *Bacillota* demonstrated the highest relative abundance, whereas *Mycoplasmatota* emerged as the most prevalent taxon in the Simple-CAP group. Specifically, *Bacillota* and *Actinomycetota* exhibited elevated relative abundances in the Asthma-CAP cohort compared to their counterparts in the Simple-CAP group, while *Mycoplasmatota* displayed reduced relative

Term	Asthma-CAP group ($n = 39$)	Simple-CAP group ($n = 39$)	*P* value
Age (month)	56.44 ± 32.872	56 ± 30.584	0.952
Sex			0.784
Male	31 (79.5%)	30 (76.9%)	
Female	8	9	
Fever	33 (84.6%)	34 (87.2%)	0.745
Days of fever before sampling	4.74 ± 4.644	6.77 ± 5.719	0.072
Cough	39 (100%)	39 (100%)	-
LOS (d)	8.18 ± 2.846	7.21 ± 3.518	0.071
CRP (mg/L)	4.65 ± 6.10	13.28 ± 30.15	0.376
WBC (*10^9/L)	9.63 ± 4.84	10.09 ± 3.86	0.204
PCT (ng/mL)	0.27 ± 0.60	1.61 ± 8.31	0.738
HBP (ng/mL)	98.28 ± 80.89	119.06 ± 96.75	0.41
ESR (mm/h)	13.21 ± 9.16	26.22 ± 21.20	0.005*
LDH (U/L)	368.82 ± 134.81	395.13 ± 190.82	0.682
D-dimer (ng/mL)	189.97 ± 132.91	663.42 ± 928.64	0.001*
Oxygen support	9 (23.1%)	24 (61.5%)	0.001*
ICU	0	2 (5.1%)	0.152

Table 1. Characteristics of participants. *Indicates a statistical difference of $P < 0.05$.

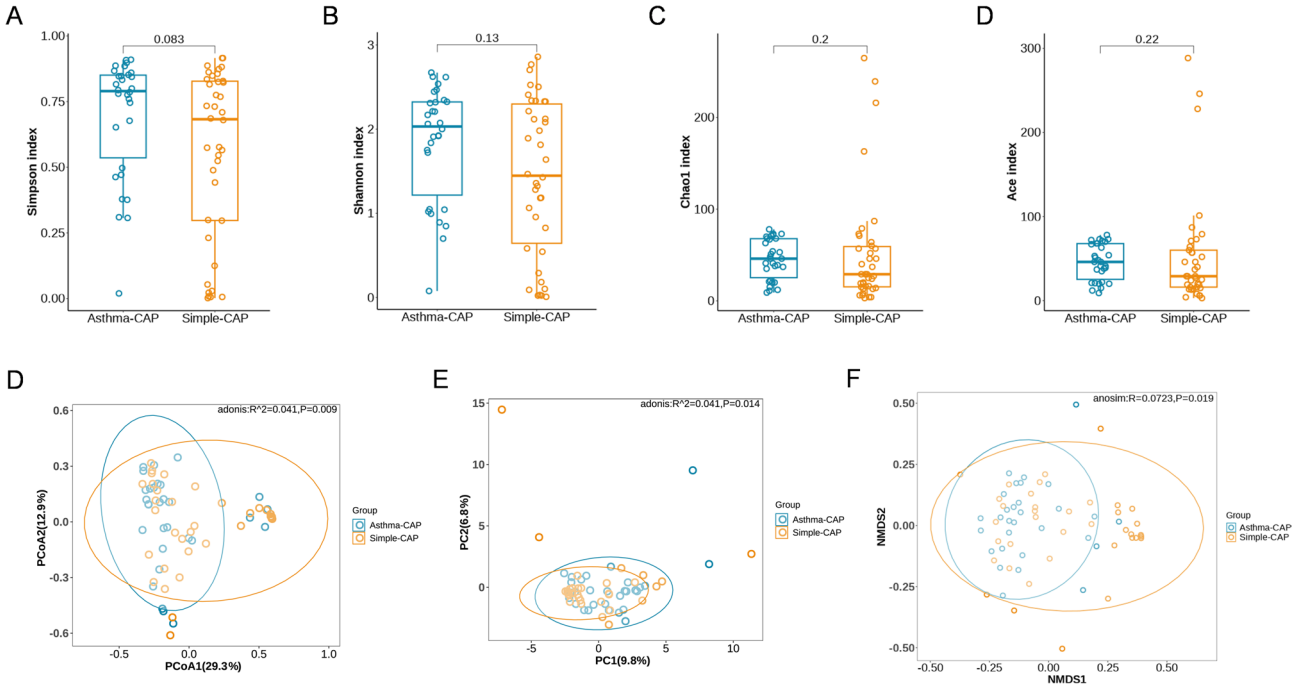

Fig. 1. Comparison of the alpha diversity and beta diversity.

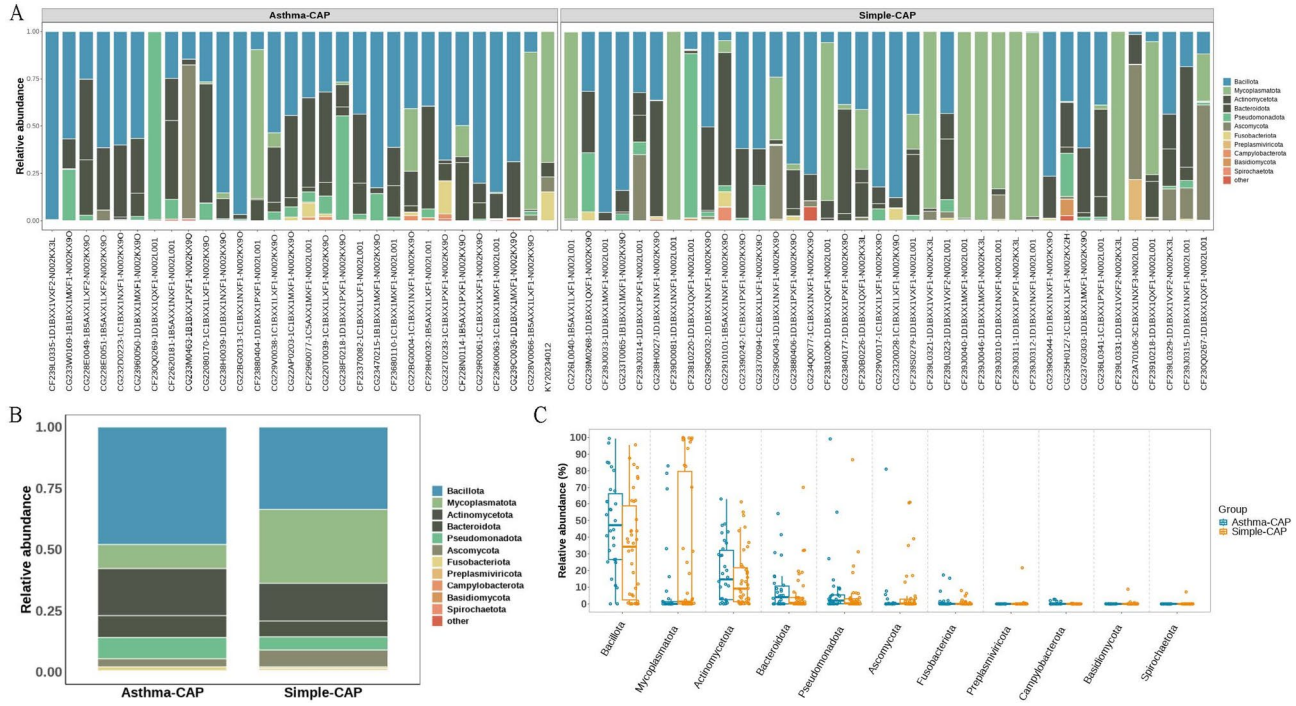

Fig. 2. Analysis of microbial composition at phylum levels.

abundances. Non-parametric Kruskal-Wallis rank-sum testing indicated no statistically significant differences in the relative abundances of these dominant microbial taxa between the two groups (Fig. 2). These findings suggest that while directional shifts in phylum-level distributions were observed, compositional variations at this taxonomic level did not attain statistical significance under nonparametric test.

Distinct genus-level microbial profiles in asthma-CAP and simple-CAP groups

In both study cohorts, the 20 most abundant microbial taxa identified at the genus level were *Streptococcus*, *Mycoplasmoides*, *Rothia*, *Actinomyces*, *Prevotella*, *Trichoderma*, *Haemophilus*, *Staphylococcus*, *Dolosigranulum*, *Veillonella*, *Corynebacterium*, *Granulicatella*, *Aureobasidium*, *Gemella*, *Moraxella*, *Mycolicibacter*, *Abiotrophia*, *Schaalia*, *Lachnoanaerobaculum* and *Fusobacterium* (Fig. 3). Within the Asthma-CAP airway microbial community, *Streptococcus* exhibited the greatest relative abundance, whereas *Mycoplasmoides* demonstrated the highest taxonomic representation in the Simple-CAP cohort. Non-parametric Kruskal-Wallis rank-sum testing revealed statistically significant differences in the relative abundances of *Streptococcus*, *Mycoplasmoides*, *Rothia*, *Trichoderma*, *Haemophilus* and *Mycolicibacter* between the two groups. Specifically, *Streptococcus*, *Rothia*, *Haemophilus*, and *Mycolicibacter* displayed elevated relative abundances in the Asthma-CAP group compared to their counterparts in the Simple-CAP group, while *Mycoplasmoides* and *Trichoderma* showed reduced taxonomic representation in the Asthma-CAP cohort (Fig. 3).

Distinct species-level microbial profiles in asthma-CAP and simple-CAP groups

To conduct a comprehensive species-level taxonomic comparison, the twenty most abundant microbial species identified were *Mycoplasmoides pneumoniae*, *Streptococcus pneumoniae*, *Streptococcus salivarius*, *Rothia mucilaginosa*, *Trichoderma citrinoviride*, *Actinomyces graevenitzii*, *Streptococcus parasanguinis*, *Staphylococcus aureus*, *Dolosigranulum pigrum*, *Prevotella histicola*, *Prevotella melaninogenica*, *Streptococcus sanguinis*, *Haemophilus influenzae*, *Streptococcus infantis*, *Prevotella jejuni*, *Actinomyces naeslundii*, *Rothia aeria*, *Streptococcus mitis*, *Veillonella atypica*, and *Mycolicibacter terrae* (Fig. 4). Notably, *Streptococcus salivarius* demonstrated the greatest relative abundance in the Asthma-CAP airway microbiome, whereas *Mycoplasmoides pneumoniae* emerged as the dominant taxon in the Simple-CAP group. Non-parametric Kruskal-Wallis rank-sum testing identified statistically significant differences in the relative abundances of *Streptococcus pneumoniae*, *Rothia mucilaginosa*, *Haemophilus influenzae*, *Mycolicibacter terrae*, *Mycoplasmoides pneumoniae* and *Trichoderma citrinoviride* between the two cohorts. Specifically, *Streptococcus pneumoniae*, *Rothia mucilaginosa*, *Haemophilus influenzae* and *Mycolicibacter terrae* displayed elevated relative abundances in the Asthma-CAP group compared to their counterparts in the Simple-CAP cohort, while *Mycoplasmoides pneumoniae* and *Trichoderma citrinoviride* showed reduced taxonomic representation in the Asthma-CAP population (Fig. 4). These findings highlight species-specific compositional disparities of the airway microbiome, with differential abundances of key respiratory pathogens and commensal organisms between the two clinical groups.

Distinct microbial biomarkers in asthma-CAP and simple-CAP groups

To further pinpoint microbial biomarkers with discriminative potential between the two cohorts, Linear Discriminant Analysis Effect Size (LEfSe) was performed on the metagenomic datasets. This analysis uncovered significant compositional divergence in airway microbial communities between the Asthma-CAP and Simple-CAP groups (Fig. 5). The LEfSe identified 26 signature taxa in the Asthma-CAP cohort, such as *Rothia mucilaginosa*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. Conversely, the Simple-CAP group exhibited 17 discriminatory microorganisms, such as *Mycoplasmoides pneumoniae*, *Trichoderma citrinoviride*,

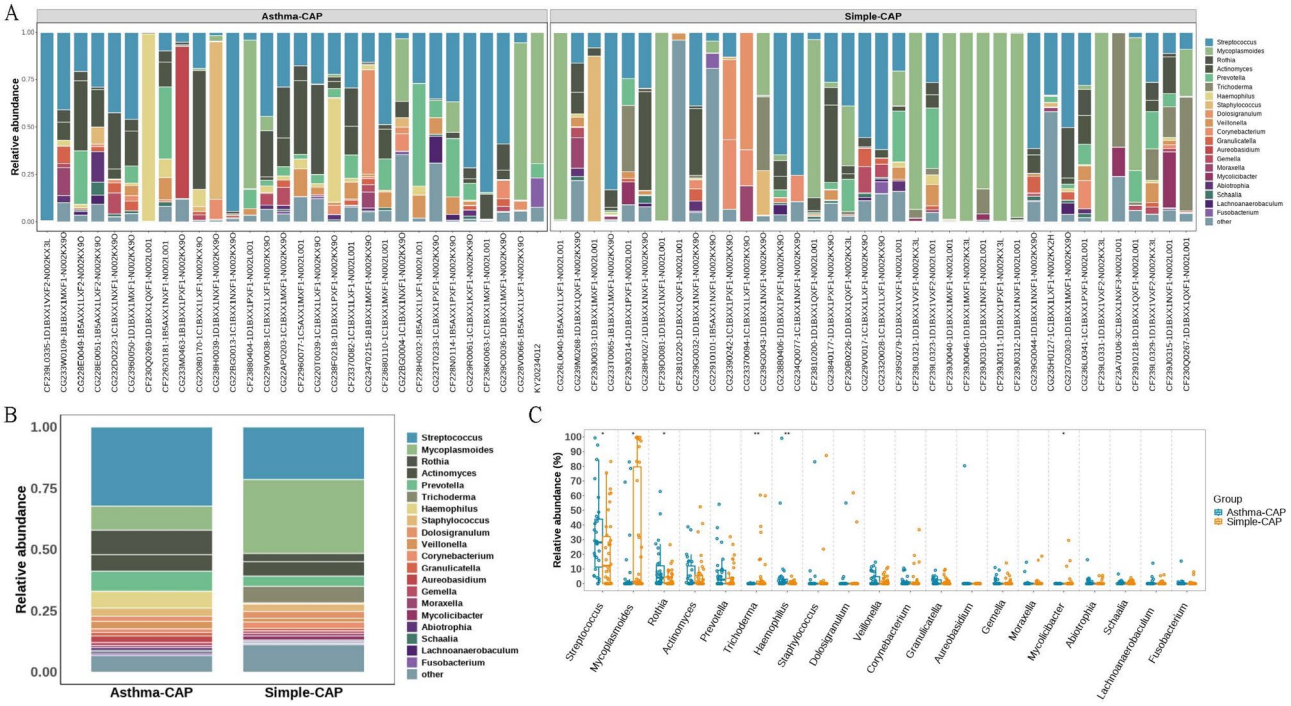

Fig. 3. Analysis of microbial composition at genus levels.

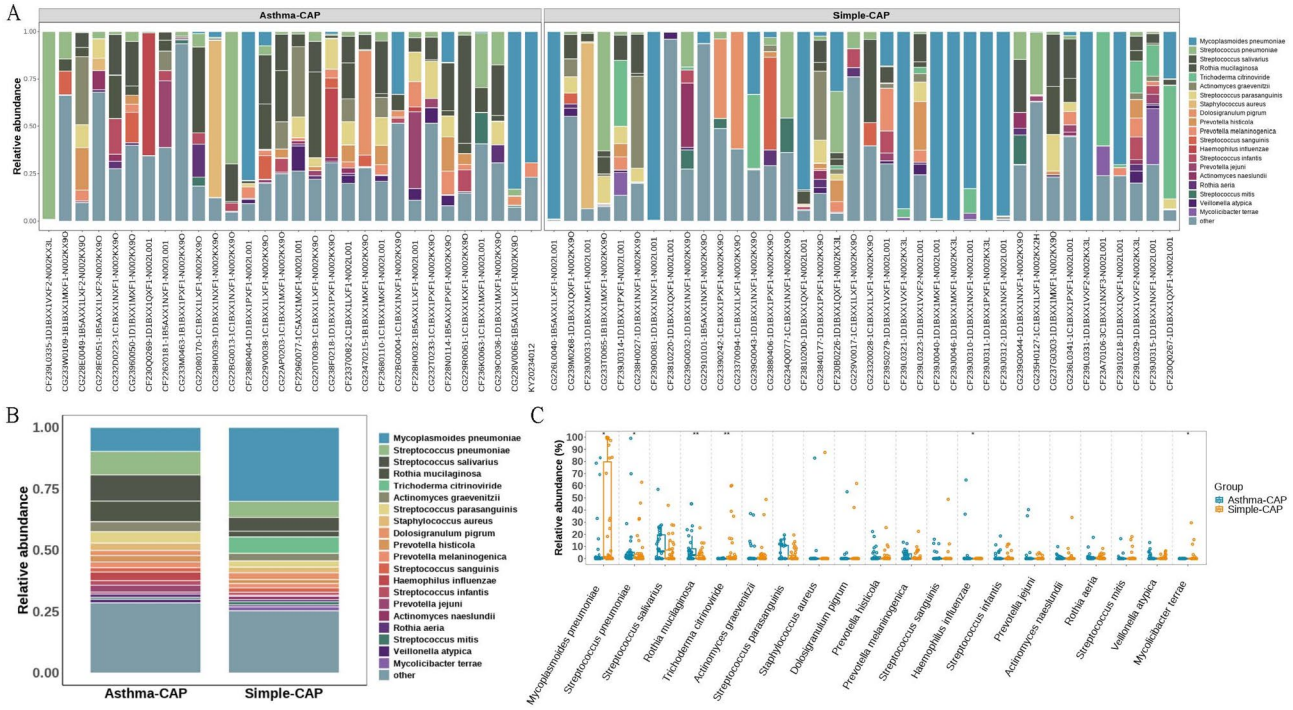

Fig. 4. Analysis of microbial composition at species levels.

Mycolicibacter terrae, *Streptococcus pseudopneumoniae* and *Acinetobacter johnsonii* (Fig. 5). These findings highlight taxon-specific microbial signatures associated with distinct clinical phenotypes, with differential enrichment of putative pathogens and commensal species between the two groups.

Correlation between microbial abundance and clinical parameters in asthma-CAP and simple-CAP patients

Spearman correlation analysis revealed significant associations between the relative abundances of microorganisms and multiple clinical parameters. Notably, *Mycoplasmoides pneumoniae* relative abundance exhibited significant positive correlations with age ($r = 0.383$, $p = 0.001$), neutrophil percentage (N%) ($r = 0.344$, $p = 0.004$), lactate dehydrogenase (LDH) levels ($r = 0.351$, $p = 0.003$), and D-dimer concentration ($r = 0.258$, $p = 0.033$), while demonstrating a significant negative correlation with lymphocyte percentage (L%) ($r = -0.333$, $p = 0.006$). Conversely, *Streptococcus pneumoniae* relative abundance showed significant negative correlations with age ($r = -0.366$, $p = 0.002$) and LDH levels ($r = -0.249$, $p = 0.041$). *Streptococcus salivarius* relative abundance demonstrated a significant negative correlation with LDH levels ($r = -0.293$, $p = 0.015$), whereas *Rothia mucilaginosa* exhibited a similar inverse association with LDH ($r = -0.243$, $p = 0.046$). Additionally, *Trichoderma citrinoviride* relative abundance was positively associated with neutrophil percentage (N%) ($r = 0.255$, $p = 0.036$), erythrocyte sedimentation rate (ESR) ($r = 0.276$, $p = 0.023$), and D-dimer concentration ($r = 0.298$, $p = 0.014$), while negatively correlated with lymphocyte percentage (L%) ($r = -0.268$, $p = 0.014$). *Actinomyces graevenitzii* relative abundance displayed a significant negative correlation with LDH levels ($r = -0.244$, $p = 0.045$). *Dolosigranulum pigrum* relative abundance, however, demonstrated significant negative correlations with hospital length of stay ($r = -0.259$, $p = 0.033$), white blood cell (WBC) count ($r = -0.314$, $p = 0.009$), and ESR ($r = -0.263$, $p = 0.030$). *Prevotella histicola* relative abundance was inversely associated with LDH levels ($r = -0.302$, $p = 0.012$). Conversely, *Prevotella melaninogenica* relative abundance exhibited significant positive correlations with age ($r = 0.259$, $p = 0.033$), neutrophil percentage (N%) ($r = 0.338$, $p = 0.005$), and high blood pressure (HBP) ($r = 0.281$, $p = 0.020$), alongside a significant negative correlation with lymphocyte percentage (L%) ($r = -0.360$, $p = 0.003$). Moreover, *Streptococcus infantis* relative abundance showed significant positive associations with neutrophil percentage (N%) ($r = 0.349$, $p = 0.004$) and significant negative correlations with lymphocyte percentage (L%) ($r = -0.361$, $p = 0.003$). *Prevotella jejuni* relative abundance demonstrated a significant positive correlation with hospital length of stay ($r = 0.275$, $p = 0.023$). Conversely, *Streptococcus mitis* relative abundance was inversely associated with age ($r = -0.345$, $p = 0.004$) (Fig. 6). These findings underscore the complex interplay between airway microbial composition and clinical phenotypes, highlighting potential microbial biomarkers associated with disease severity and immune response parameters.

Discussion

Our study analyzed metagenomic next-generation sequencing results of nasopharyngeal aspirates from 78 patients. The findings revealed differences in airway dominant pathogens and microbial community structure between children with asthma complicated by pneumonia and those with simple CAP. Notably, *Streptococcus*

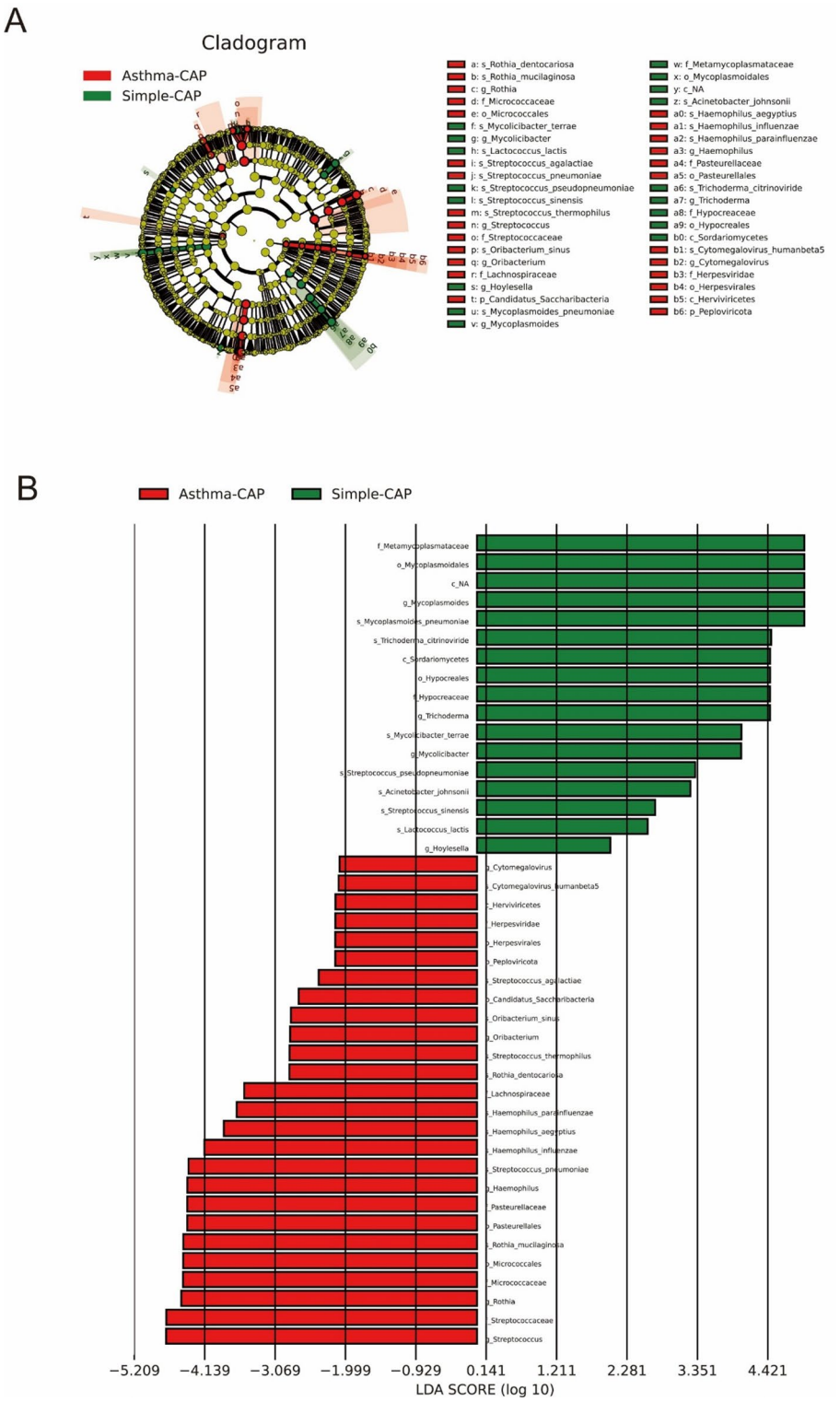

Fig. 5. Multi-level species difference discriminant analysis.

pneumoniae and *Rothia mucilaginosa* dominated in the Asthma-CAP group, whereas *Mycoplasmoides pneumoniae* and *Trichoderma citrinoviride* were enriched in the Simple-CAP group. These differences in microbial composition may reflect the changes of immune-microbial interactions in patients' airways, which potentially affects the progress and clinical outcomes of the disease. Our results are consistent with emerging evidence that respiratory microbiota may contribute to the clinical course of various pulmonary diseases by altering host immune responses or the virulence of potential pathogens in a synergistic or additive manner[13].

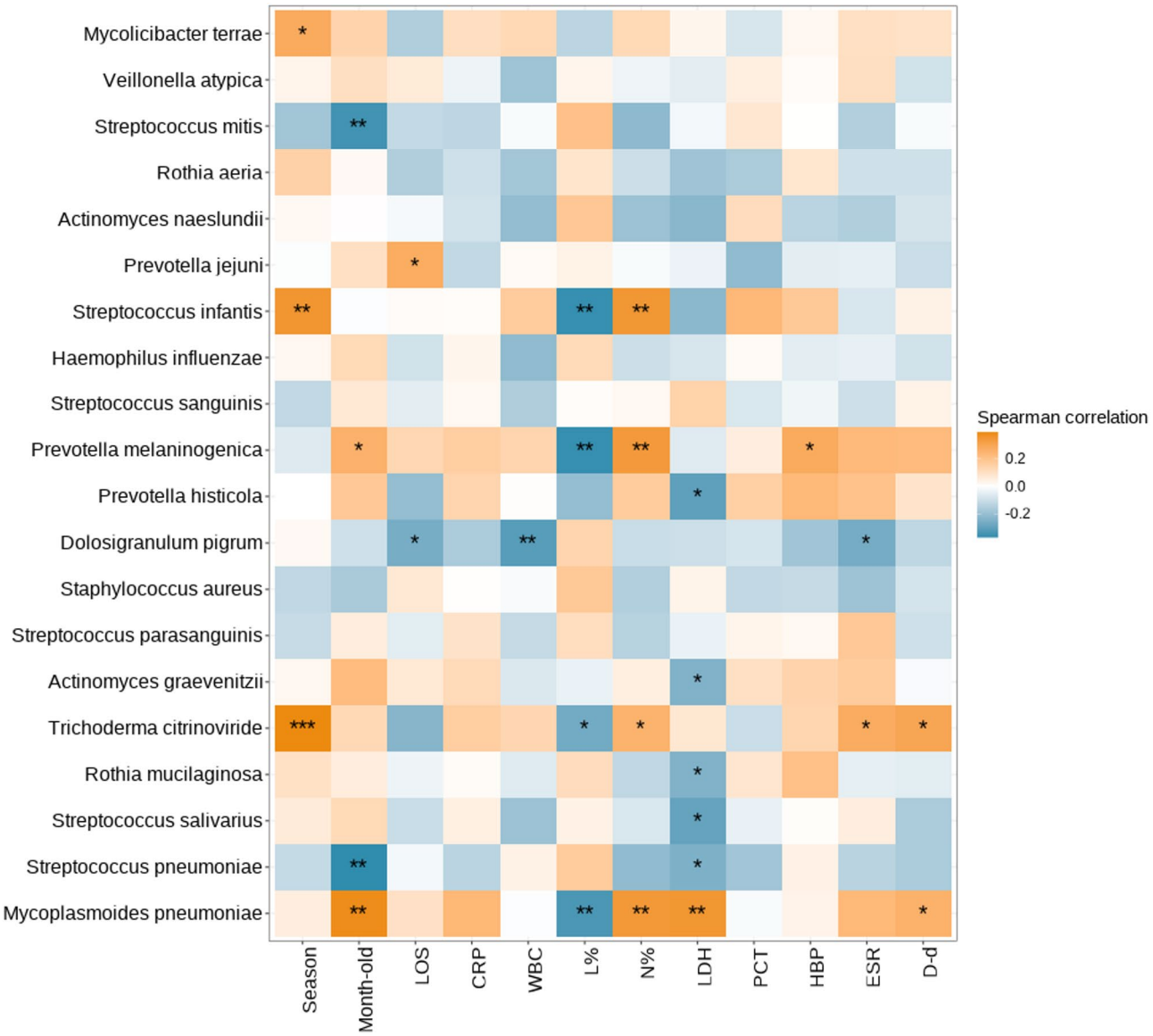

Fig. 6. Correlation analysis between different species and clinical indicators.

Traditional CAP pathogenesis posits that a single pathogen invades and proliferates in the respiratory tracts[4,14]. However, a newer model suggests that CAP was due to respiratory microecological imbalance[15]. The composition of respiratory microflora influences the clinical course of CAP[13]. Our research supports this model, identifying multiple pathogens in the respiratory tracts of CAP children. Moreover, *Mycoplasmoides pneumoniae* predominance in Simple-CAP aligns with its recognized role in pediatric CAP, particularly in children aged five years and older[4,16]. Notably, while *Trichoderma* is rarely associated with invasive pulmonary fungal disease in immunocompetent hosts, its recurrent detection in asthmatic patients with CAP warrants further investigation. Potential explanations include environmental exposure (e.g. mold in residential settings) or transient colonization, rather than active infection, as no patients exhibited radiological or serological evidence of fungal pneumonia. Compared with the existing studies, the advantages of *Streptococcus pneumoniae* in asthma-CAP patients are similar to those found in chronic obstructive pulmonary disease, where *Streptococcus* are frequently enriched during exacerbations, then increase airway inflammation and obstruction, sputum production, and bronchoconstriction[17,18]. The increased abundance of *Rothia mucilaginosa* in asthmatic children could signify its dual capacity as both commensal and pathobiont, as seen in cystic fibrosis, where its abundance correlates with airway inflammation[19,20]. These parallels highlight shared mechanisms of microbial dysbiosis in chronic respiratory diseases, where specific taxa drive inflammation or impair epithelial barrier function.

Dysbiosis in the pulmonary microbiome, which imbalances the composition and size of the lung microbiome, is increasingly implicated in chronic pulmonary conditions. In COPD, chronic airway inflammation is related to the microorganism group dominated by *γ-proteobacteria*, and the severity of the disease is related to the diversity of microorganisms[21]. Similarly, in sarcoidosis, an overrepresentation of *propionibacterium* and *mycobacteria* has been linked to granulomatous inflammation[22].

Term		Asthma-CAP group	Simple-CAP group	*P* value
Age (month)	Before PSM	58.39 ± 33.23	71.71 ± 35.14	0.028*
	After PSM	56.44 ± 32.87	56.00 ± 30.58	0.952
Sex	Before PSM			0.016*
Male		31 (75.6%)	102 (55.1%)	
Female		10	83	
Sex	After PSM			0.784
Male		31 (79.5%)	30 (76.9%)	
Female		8	9	
Days of fever before sampling	Before PSM	4.90 ± 4.59	8.25 ± 6.06	< 0.001*
	After PSM	4.74 ± 4.64	6.77 ± 5.719	0.072

Table 2. PSM parallel hypothesis testing. *Indicates a statistical difference of $P < 0.05$.

To explore the relationship between airway microecological disruption and clinical indicators, we analyzed correlations between different species and clinical metrics in both groups. Our findings in Asthma-CAP, especially the enrichment of *Streptococcus pneumoniae*, may induce chronic inflammatory process by activating Th2-biased airway inflammation and other ways to aggravate the progress of asthma[23]. Furthermore, the correlation between *Mycoplasmoides pneumoniae* abundance and inflammatory markers (N%, LDH, D-dimer) mirrors observations in refractory mycoplasma pneumonia[24–26]. *Mycoplasmoides pneumoniae* infection can induce macrophages to produce TNF-α, increasing inflammatory cell infiltration in the lung and stimulating lymphocytes and macrophages to produce and release additional cytokines, which exacerbates tissue damage[27]. These examples highlight the bidirectional interaction between microbial communities and host immune responses in influencing disease progression.

This investigation has several inherent limitations. First, the relatively small sample size and single-center recruitment strategy may restrict the generalizability of the findings to broader pediatric populations. Second, while nasopharyngeal aspirates are widely utilized in pediatric respiratory research[10]these specimens may not fully recapitulate the microbial composition of the lower airways. Third, the cross-sectional nature of the study design hinders the establishment of temporal associations between microbiota alterations and clinical outcomes. Future research should incorporate longitudinal sampling to characterize dynamic microbial-host interactions over time. Additionally, integrating multi-omics approaches (e.g., metabolomics, transcriptomics) with metagenomic data could provide mechanistic insights into microbiota-driven pathogenic pathways. Environmental factors such as air pollution exposure, antibiotic use patterns, and socioeconomic determinants should also be systematically evaluated to comprehensively characterize microbiota-modulating influences. Moreover, extracting metagenome-assembled genomes (MAGs) from sequencing datasets would enable high-resolution reconstruction of microbial genomes, facilitating in-depth functional annotation and ecological network analysis. Such advancements could uncover strain-specific virulence factors or metabolic capabilities that contribute to differential disease phenotypes.

Collectively, this investigation elucidates distinct airway microbial signatures in children with asthma-complicated pneumonia versus simple CAP, highlighting the role of microbiota dysbiosis in driving respiratory disease heterogeneity. These findings converge with emerging evidence associating microbial imbalance with chronic respiratory disorders such as chronic obstructive pulmonary disease (COPD) and sarcoidosis. By integrating microbial profiling with clinical phenotypes, this work provides a foundation for precision antimicrobial strategies and microbiota-targeted therapies tailored to high-risk pediatric populations.

Methods and materials

Subject enrollment

This research was conducted from June 2022 to October 2023 at the Children's Hospital of Nanjing Medical University. Hospitalized children diagnosed with CAP were prospectively recruited, and nasopharyngeal aspirate specimens were collected for metagenomic next-generation sequencing (mNGS). A total of 41 pediatric patients with bronchial asthma, who met the inclusion criteria and had complete clinical datasets, were enrolled in the study. Using propensity score matching (PSM), variables selected for covariate balancing included patient age, sex, and fever duration before sampling. CAP patients without underlying comorbidities were matched in a 1:1 ratio, yielding 39 matched pairs assigned to the Asthma-CAP group and Simple-CAP group, respectively. Following PSM, no statistically significant differences remained in age, sex, or fever duration before sampling between the two groups, indicating that the PSM model satisfied the balance hypothesis for baseline covariates (Table 2).

The Asthma-CAP group comprised pediatric patients aged 0 to 14 years meeting diagnostic criteria for CAP and bronchial asthma, including cases of cough variant asthma (CVA). Conversely, the Simple-CAP group consisted of age-matched children (0–14 years) with confirmed CAP diagnosis but without a prior history of bronchial asthma or related allergic respiratory conditions.

Exclusion criteria encompassed individuals with preterm birth before 32 weeks of gestation, a history of bronchopulmonary dysplasia, diabetes mellitus with active infections, primary immunodeficiency disorders, or severe cardiovascular, neurological, hepatic, renal, or other vital organ dysfunction. Additionally, individuals unable to cooperate with the investigation or provide airway samples were excluded from the study.

Sample collection
Nasopharyngeal aspirates were collected from patients following standard procedures: On the day of admission, nasopharyngeal aspirates were collected by using sterile negative pressure suction devices, placed in sterilized tubes, and immediately sent to the laboratory for dilution and subsequent sequencing analysis.

Sample processing and sequencing
For DNA extraction, nasopharyngeal aspirate was liquefied using 0.1% DTT (dithiothreitol) for 20 min at 56 °C prior to extraction. The quantity of DNA was assessed using the Qubit fluorometer (Thermo Fisher Scientific), while the quality was evaluated using the NanoDrop spectrophotometer (Thermo Fisher Scientific). For RNA extraction, the QIAamp Viral RNA Mini Kit (Qiagen) was employed to extract RNA from nasopharyngeal aspirates. RNA samples were quantified using the Qubit fluorometer before library construction.

DNA libraries were prepared using the Hieff NGS C130P2 OnePot II DNA Library Prep Kit for MGI (Yeasen Biotechnology) according to the manufacturer's protocols. The rRNA was removed from the total RNA, and the library was constructed after purification.Agilent 2100 was used for quality control and DNA libraries were 50 bp single-end sequenced on MGISEQ-200.

Bioinformatics analysis
We use in-house developed bioinformatics pipeline for pathogen identification. Briefly, high-quality sequencing data were generated by removing low quality reads, adapter contamination, duplicated and short (length < 36 bp) reads. Human host sequences were identified by mapping to human reference genome (hs37d5) using bowtie2 software (version 2.2.6). Reads that could not be mapped to the human genome were retained and aligned with microorganism genome database for pathogens identification. Our microorganism genome database contained bacteria, fungi, virus and parasite genomic sequences (download from https://www.ncbi.nlm.nih.gov/).

Microbial flora analysis
Statistical analysis was performed by R software (version 4.0.1). Alpha diversity was estimated by the Shannon index, the Simpson index, the ACE index and the Chao1 index based on the taxonomic profile of each sample, beta diversity was assessed by the Bray-Curtis measure, and compared between Asthma-CAP and Simple-CAP patients by using Wilcoxon rank sum test, and was subsequently visualized by principal coordinate analysis (PCoA) plot, principal component analysis (PCA) plot and Non-metric multidimensional scaling (NMDS) plot. PERMANOVA was performed by the R package "vegan" to analyze Bray-Curtis distance in different Asthma-CAP and Simple-CAP groups. Differential relative abundance of taxonomic groups at each taxonomic level among groups was tested by using Kruskal-Wallis rank sum test (R package "kruskal.test"). The species with mean relative abundances greater than 0.1% and penetrance greater than 10% among all samples were compared. Spearman's correlations between clinical features and the relative abundances of species were calculated by the R package "cor.test", and FDR correction was adopted to adjust all p values. Statistically significant differences in the relative abundance of microbe among groups were assessed by the linear discriminant analysis of effect size (LEfSe) analysis.

Statistical analysis
Statistical analyses and propensity score matching were performed using SPSS26.0.

Data availability
The pathogen reads of our study were deposited in the Genome Warehouse in the China National Center for Bioinformation under project CRA022727 and are available at the following URL: https://ngdc.cncb.ac.cn/gsa/search?searchTerm=CRA022727.

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Author contributions

Lei Chen and Huan Chen wrote the main manuscript text, Lv and Guo prepared Tables 1, 2 and 3, Wu prepared Figs. 1, 2, 3 and 4, all authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Informed consent

All methods were carried out in accordance with relevant guidelines and regulations. All experimental protocols were approved by Ethics Committee of Children's Hospital of Nanjing Medical University (202207144-1). Informed consent was obtained from all subjects and their legal guardians.

Additional information

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