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Characterization of the oral mycobiome of Portuguese with allergic rhinitis and asthma

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ABSTRACT

Allergic rhinitis and asthma are two prevailing chronic airway diseases and serious public health concerns. Previous research has already described the role of the airway bacteriome in these two diseases, but almost no study so far has explored the mycobiome and its possible association to airway inflammation. Here we sequenced the internal transcribed spacers (ITS) 1 and 2 to characterize the oral mycobiome of 349 Portuguese children and young adults with allergic rhinitis alone (AR) or with asthma (ARAS), asthmatics (AS) and healthy controls (HC). Our genomic analyses showed that the two most abundant fungal phyla (Ascomycota and Basidiomycota) and 3–5 of the 14 most abundant fungal genera (*Cladosporium, Aspergillus, Aleurina, Candida* and *Rhodotorula*) in the mouth differed significantly ($P \leq 0.04$) between both rhinitic groups and HC. However, none of the same taxa varied significantly between the three respiratory disease groups (AR, ARAS and AS). The oral mycobiomes of respiratory ill patients showed the highest intra-group diversity (microbial richness and evenness), while HC showed the lowest, with all alpha-diversity indices varying significantly ($P \leq 0.0424$) between them. Similarly, all disease groups showed significant differences ($P \leq 0.0052$) in microbial structure (i.e., beta-diversity indices) when compared to HC samples. Thirty metabolic pathways (PICRUSt2) were differentially abundant (Wald's test) between AR or ARAS and HC patients, but only one of them (D-galactose degradation I) was over abundant (log2 Fold Change >0.75) in the ARAS group. Spiec-Easi fungal networks varied greatly among groups, which suggests chronic respiratory allergic diseases may alter fungal connectivity in the mouth. This study increases our comprehension of the role of the oral mycobiome in allergy-related conditions. It shows for the first time that the oral mycobiota changes during health and allergic rhinitis (with and without asthma comorbidity) and highlights specific taxa, metabolic pathways and fungal interactions that may relate to chronic airway disease.

1. Introduction

Allergic rhinitis and asthma are two prevailing chronic airway diseases in many developed countries, where they cause significant health and economic stress to their governments and individuals (Sa-Sousa et al., 2012; Todo-Bom et al., 2007; Fonseca et al., 2021). In

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Portugal allergic rhinitis has a prevalence of 26.1 % in adults and 9 %–10 % in children and adolescents (Todo-Bom et al., 2007; Falcão et al., 2008; Muc et al., 2014). Similarly, asthma impacts about 695,000 Portuguese and has a prevalence of 6.8 % in adults and 8.4 % in children and adolescents (Sa-Sousa et al., 2012; Muc et al., 2014; Ferreira-Magalhaes et al., 2016).

Allergic rhinitis is a Th2 type of inflammation of the nasal mucosa, characterized by sneezing, congestion, itching and rhinorrhea (Savoure et al., 2022; Steelant et al., 2016, 2018; Acevedo-Prado et al., 2022). While asthma is considered a multifactorial condition of the airways characterized by chest tightness, with bronchial obstruction, inflammation and mucous production (Dharmage et al., 2019; Mims, 2015; Licari et al., 2018). Allergic rhinitis and asthma frequently coexist (Compalati et al., 2010; Bousquet et al., 2019; Ferreira-Magalhaes et al., 2015; Pite et al., 2014; Small et al., 2018), which indicates that they may constitute a combined airway inflammatory syndrome with multiple epidemiological, pathophysiological and clinical connections (Compalati et al., 2010; Bergeron and Hamid, 2005; Kim et al., 2008; Pawankar, 2006); i.e., the so called concept of a united airway disease (Bousquet et al., 2023). In Portugal, about 46 % of the individuals with asthma also show allergic rhinitis (Acevedo-Prado et al., 2022; Valero et al., 2009).

Multiple studies of the nasal and oral (less explored) cavities have shown that the bacterial communities living on them act as gatekeepers of respiratory health (Man et al., 2017), playing a major role in the emergence, development and severity of both allergic rhinitis (Bender et al., 2020; Chen et al., 2022; Gan et al., 2021; Lal et al., 2017; Kim et al., 2022; Azevedo et al., 2023; Pérez-Losada et al., 2023a, 2023b) and asthma (Huang and Boushey, 2014, 2015; Brar et al., 2012; Dickson and Huffnagle, 2015; Castro-Nallar et al., 2015; Teo et al., 2015; Bogaert et al., 2011; Pérez-Losada et al., 2017, 2018, 2015, 2016a, 2016b; Raita et al., 2021; Dinwiddie et al., 2018; Hufnagl et al., 2020; Losol et al., 2021; Frati et al., 2018). However, the role of the fungal microbiome (i.e., mycobiome) in respiratory health is less understood (de Dios Caballero et al., 2022; Nguyen et al., 2015; Li et al., 2024; Oliveira et al., 2023). Emerging evidence has already stated the importance of the lower airways mycobiome in asthma (Nguyen et al., 2015; van Tilburg Bernardes et al., 2020; Carpagnano et al., 2016; Goldman et al., 2018; Rick et al., 2020), but little to nothing is known about the contribution of the fungal communities of the upper respiratory tract to chronic respiratory disease (Oliveira et al., 2023; van Tilburg Bernardes et al., 2020; Goldman et al., 2018; Rick et al., 2020) – although its diversity and relevance have been already put forward (Diaz, 2021; Bandara et al., 2019). Two studies so far have shown that nasal fungi are implicated in the onset and development of asthma (Yuan et al., 2023) and allergic rhinitis (Jung et al., 2015) in susceptible individuals; but, up to our knowledge, the contribution of the mycobiome of the mouth to rhinitis and asthma remains unexplored.

Here we have applied amplicon (ITS1-ITS2) next-generation sequencing to 349 mouth swabs from Portuguese children and adults with allergic rhinitis (with and without asthma comorbidity), asthma and healthy controls to characterize their oral mycobiomes. We reveal how the composition, structure, interactions and metabolic functions of their fungal communities vary across these four clinical groups.

2. Materials and methods

2.1. Participants

All participants enrolled in this study were part of the ASMAPORT Project (PTDC/SAU-INF/27,953/2017). We obtained written consent from all of them or their legal guardians using the informed consent documents approved by the ethics committee. This study was approved by the "Comissão de Ética para a Saúde" (Parecer_58–17, March 2017) of the Centro Hospitalar Universitário São João, Facultade de Medicina (Porto, Portugal).

ASMAPORT was a cross-sectional study of Portuguese adults and children created to investigate host-microbe during asthma and rhinitis. Participants were recruited from northern Portugal while attending the outpatient clinic of the Serviço de Imunoalergologia in the Centro Hospitalar Universitário São João from July 2018 to January 2020. Healthy volunteers from the Porto area with no history of respiratory illness were also enrolled in the study but did not fill out the questionary or facilitated clinical information. The diagnosis of allergic rhinitis was corroborated by an allergy specialist based on a specific IgE or positive skin test to at least one standard inhalant allergen in Portugal and available clinical information (Bousquet et al., 2009; Pereira et al., 2006). Asthma diagnosis was determined by the attending physician according to the observed typical symptoms in the previous twelve months before sampling or a positive bronchodilator responsiveness test with salbutamol (Silva et al., 2019). Further details are provided in Pérez-Losada et al. (2023a, 2023b).

2.2. Sampling

A total of 349 adults and children (36 and 313, respectively) participated in this study (Table S1). Individuals were classified into four clinical groups: allergic rhinitis (AR = 47), allergic rhinitis with asthma (ARAS = 161), asthma (AS = 12) and healthy controls (HC = 129 individuals). Oral samples were collected by swabbing the buccal mucosa of the left and right cheeks during 30 s with the same cotton swab. Further detail is provided in Pérez-Losada et al. (2023a, 2023b). Since the sample size of the AS group was small (12 participants), we have only used this group in some analyses and applied statistical tests that account for small sample sizes (see below).

2.3. ITS high-throughput sequencing

Total DNA was extracted from swabs using the ZymoBIOMICS™ DNA Miniprep Kit D4300. DNA extractions were sequenced using the Schloss' MiSeq_WetLab_SOP protocol in Kozich et al. (2013). DNA samples were amplified and sequenced for the internal transcribed spacers (ITS) 1 and 2 regions (~230 bp) following the Earth Microbiome Project's protocols (Thompson et al., 2017) and primers ITS1F Fwd: CTTGGTCATTTAGAGGAAGTAA and primer ITS2 Rev: GCTGCGTTCTTCATCGATGC – https://earthmicrobiome.org. All samples were sequenced in a single sequencing run of the Illumina MiSeq at the University of Michigan Medical School. Negative controls processed as indicated above displayed no PCR band on an agarose gel. We used five mock communities (i.e., reference samples with a known composition) and eight water and reagent negative controls to detect reagents contaminated with microbial DNA and measure sequencing error rate. We did not detect evidence of contamination and our sequencing error rate was 0.0053 %.

2.4. Mycobiome analyses

ITS amplicon sequence variants (ASV) in each sample were inferred using dada2 version 1.18 (Callahan et al., 2016) and following author's recommendations for the ITS region (https://benjjneb.github.io/dada2/ITS_workflow.html). Reads were filtered using standard parameters, with no uncalled bases, maximum of 2 expected errors and truncating reads at a quality score of 2 or less. Forward and reverse reads were merged, and chimeras were identified. Taxonomic assignment was performed against the UNITE v9.0 2023–07–18 database (Nilsson et al., 2019) using the implementation of the RDP naive Bayesian classifier available in the dada2 R package (Quast et al., 2013; Wang et al., 2007). ASV sequences were aligned in MAFFT (Katoh and Standley, 2013) and used to build a tree with FastTree (Price et al., 2010). Phylogenetic information and ASV tables were imported into phyloseq (McMurdie and Holmes, 2013) for further analysis. ITS files, clinical metadata and BioSample attributes for all samples in this study have been deposited in

the NCBI (PRJNA1107919). Metadata information and ASV counts with their corresponding taxonomy are presented in Tables S1 and S2, respectively.

Samples were normalized using the negative binomial distribution approach (McMurdie and Holmes, 2014) as indicated in the package DESeq2 (Love et al., 2014). Taxonomic and phylogenetic alpha-diversity (within sample) were estimated using Chao1 richness and Abundance-based Coverage Estimator (ACE), Shannon and Phylogenetic diversity indices. Beta-diversity (between-sample) was estimated using Bray–Curtis, Jaccard and phylogenetic Unifrac (unweighted and weighted) distances, while dissimilarity between samples was explored using principal coordinates analysis (PCoA).

Differences in fungal phyla and genera composition and alpha-diversity between disease groups (AR, ARAS and AS) and healthy individuals (HC) were assessed using linear models (mixed and standard) analysis to account for the non-independence of subjects (random effect) – lmer4 R package (Bates et al., 2015). We also included age, season of the year and sex as covariables in all our initial model comparisons. Linear models with randomized subjects were not better than those without random effects, as suggested by their similar or lower scores for the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC); hence we did not use random effects in our final linear models. Additionally, none of the covariables were significant for any of the taxonomic and diversity indices compared. Beta-diversity indices were compared using permutational multivariate analysis of variance (adonis) – vegan R package (Dixon, 2003). The Benjamini–Hochberg method at alpha = 0.05 (Benjamini and Hochberg, 1995; Cook, 1977) was applied to correct for multiple hypotheses testing. All the analyses were performed in R (Team RDC, 2008) and RStudio (RStudio RT, 2015).

2.5. Functional analyses

Metabolic pathways were predicted by imputation of gene families and genomes as implemented in PICRUSt2 (Douglas et al., 2020). Briefly, we used the fungi ITS reference database provided by the developers to align our ITS sequences (minimum alignment 0.6) and then place them onto an ITS phylogenetic tree. Using ASV abundances obtained in dada2, we predicted gene family profiles and ultimately sample pathway abundances. Pathways were annotated using the MetaCyc database (Caspi et al., 2020) and differential pathway abundance among groups was determined in DESeq2 based on the Wald test (adjusted p-value <0.01). Statistical analyses and visualization were conducted using functions in the ggpicrust R package (Yang et al., 2023).

2.6. Network analyses

To reveal changes in fungal community structure, we used covariation network analysis as implemented in Spiec-Easi (Kurtz et al., 2015). We estimated networks for AR, ARAS and HC at the genus level (abundance filter threshold = 0.0005; mb method; greedy clustering). Network estimation, statistics, and visualization was carried out in the microeco R package (Liu et al., 2021).

3. Results

We collected oral swabs from a cohort of 349 participants (220 individuals with respiratory disease and 129 healthy controls) from northern Portugal comprised mainly of young adults and children (Table S1). The median age of the participants was 12.7 ± 5.5 years and 54.2 % were female. Individuals with respiratory disease were classified into three groups: AR (Hufnagl et al., 2020), ARAS (161) and AS (12 subjects). We sequenced the ITS1-ITS2 gene to characterize the oral mycobiome of each participant. Seventy-four samples (i.e., technical replicates) from the following groups were sequenced twice due to seemingly faint PCR bands in agarose gels: AR (19 samples), ARAS (50 samples), AS (4 samples) and HC (1 samples). ASV singletons and samples with <1030 reads were eliminated, rendering a final data set of 392 samples from 323 participants with the following distribution: AR (62 samples from 44 individuals), ARAS (203 samples from 156 individuals), AS (16 samples from 12 individuals) and HC (111 samples from 111 individuals).

3.1. Mycobiome taxonomic diversity and structure

Our oral mycobiome (392 samples after quality control) dataset comprised 8360,575 clean reads, ranging from 1030 to 139,338 sequences per sample (mean = 21,328.0) and a total of 6263 ASVs (Table S2). AR samples had 1085 unique ASVs, ARAS samples had 2681, AS samples had 296 and HC samples had 1138 (Fig. S1). The four groups shared 104 ASVs, the disease groups shared 143 ASVs, while other pairs and trios of groups shared a variable number, ranging from 4 to 355 ASVs (Fig. S1).

The oral mycobiome sequences across all 392 filtered samples were classified into two dominant (<1 % abundance) Phyla: Ascomycota (65.1 %) and Basidiomycota (33.5 %) (Table 1). Those Phyla comprised 14 dominant (<1 %) genera (Table 1 and Fig. 1), being the most abundant *Aleurina* (16.4 %), *Cladosporium* (13.8 %), *Candida* (7.8 %) and *Rhodotorula* (6.7 %). All the other detected phyla and genera accounted for <1 % of the total ITS sequences each. No core microbiome (prevalence <90 %) was detected for the respiratory disease or healthy patients.

We compared the mean relative abundance of specific taxa in subjects with respiratory disease and healthy controls. The two dominant fungal phyla (Ascomycota and Basidiomycota) comprising the oral microbiome showed significant differences (LME model test; $P \leq 0.0246$) in their mean relative proportions between AR or ARAS and HC (Table 1). Of the 14 dominant fungal genera comprising the oral microbiome (Fig. 1 and Table 1), three and five genera (*Cladosporium, Aspergillus, Aleurina, Candida* and *Rhodotorula*) showed significant differences in their mean relative proportions between AR-HC and ARAS-HC after FDR correction, respectively. None of the dominant fungal phyla and genera varied significantly between AS and HC or the three respiratory disease groups (Table 1).

Alpha-diversity indices (Shannon, Chao1, ACE, and PD) of microbial community evenness and richness varied among clinical groups (Fig. 2 and Table S3). Respiratory disease groups displayed the highest diversity for all indices, while HC showed the lowest. ARAS, AR and AS versus HC comparisons were significantly distinct (LME model test; $P \leq 0.0068$) for all indices but PD, which only varied significantly for AR-HC (LME model test; $P = 0.0424$). All the other pairwise comparisons were not significant.

To characterize the structure of the oral mycobiomes (beta diversity), we applied principal coordinates analysis (PCoA) to Bray–Curtis, Jaccard and Unifrac (unweighted and weighted) distance matrices. All the PCoAs showed noticeable separation of the mycobiotas from each clinical group (Fig. 3). Group dissimilarity was then confirmed by the adonis test, which detected significant differences ($P \leq 0.0052$) in beta-diversity between each of the respiratory disease groups (AS, AR and ARAS) and the healthy controls for all the distances except for AS-HC and Unifrac weighted (Fig. 3). This suggests that the mycobiomes of those patients may differ from those of healthy individuals in a similar compositional manner. Moreover, three of the nine pairwise comparisons between respiratory disease groups also resulted significant ($P \leq 0.0345$).

3.2. Mycobiome functional diversity

We inferred the functional potential of the mycobiomes of the AR, ARAS, and HC groups. We found significant differences (adjusted p-value <0.01) in abundance in 30 pathways (MetaCyc annotated)

Table 1
Mean relative proportions (%) of fungal phyla and genera in the oral mycobiome of participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS), asthma (AS) and healthy controls (HC). p-values for significant pairwise comparisons (linear model test) between groups are also displayed. ns=not significant.

		Mean relative proportions (%)				Linear model test significance					
	All	AR	ARAS	AS	HC	AR-HC	ARAS-HC	AS-HC	AR-ARAS	AR-AS	ARAS-AS
Phylum											
Ascomycota	65.11	53.53	65.95	66.17	70.99	0.0109	ns	ns	ns	ns	ns
Basidiomycota	33.53	45.19	32.92	32.25	27.14	0.0066	0.0246	ns	ns	ns	ns
Genus											
Malassezia	1.48	1.75	1.59	1.61	1.05	ns	ns	ns	ns	ns	ns
Alternaria	3.68	3.07	4.56	1.79	2.61	ns	ns	ns	ns	ns	ns
Cladosporium	13.76	13.93	17.03	13.02	7.04	0.0151	0.0002	ns	ns	ns	ns
Penicillium	1.4	1.06	1.06	0.29	2.53	ns	ns	ns	ns	ns	ns
Aspergillus	3.32	3.16	2.87	0.81	4.85	ns	0.0381	ns	ns	ns	ns
Candida	7.75	4.52	6.37	13.04	11.81	ns	0.013	ns	ns	ns	ns
Aleurina	16.4	6.44	12.74	22.01	29.64	0.0226	0.0367	ns	ns	ns	ns
Debaryomyces	1.98	3.98	2.14	0.23	0.62	ns	ns	ns	ns	ns	ns
Wallemia	3.47	4.5	4.16	0.68	1.86	ns	ns	ns	ns	ns	ns
Rhodotorula	6.72	12.42	7.32	4.53	2.05	0.0005	<0.0001	ns	ns	ns	ns
Vishniacozyma	1.33	1.86	1.01	2.71	1.38	ns	ns	ns	ns	ns	ns
Saccharomyces	4.33	5.08	5.35	0.28	2.47	ns	ns	ns	ns	ns	ns
Filobasidium	1.39	2.26	1.39	1.87	0.71	ns	ns	ns	ns	ns	ns
Agaricus	2.2	0.51	0.06	0.01	8.17	ns	ns	ns	ns	ns	ns

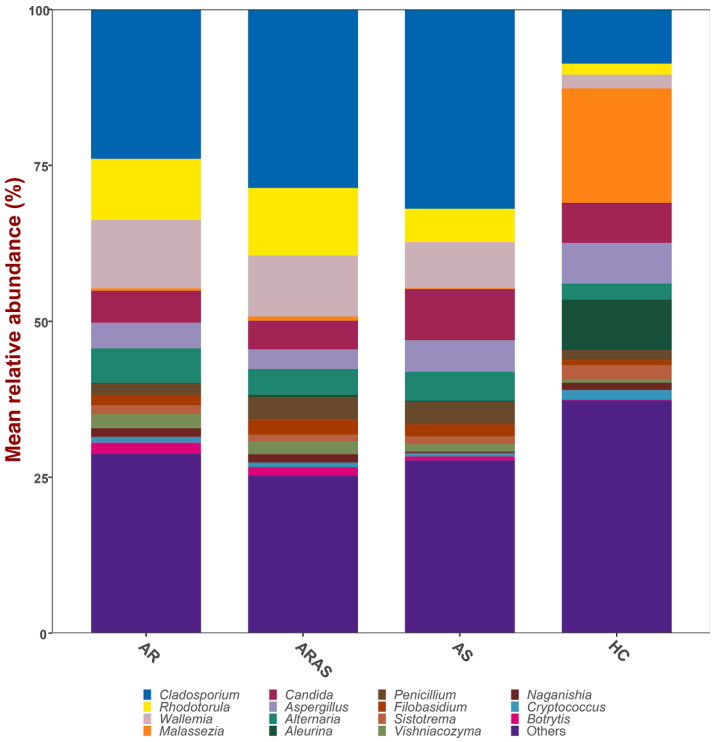

Fig. 1. Bar plots of mean relative proportions of the top fungal genera in the oral cavity of participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS), asthma (AS) and healthy controls (HC).

between AR and HC or ARAS (Fig. 4). Most changes in pathway abundance represented pathways enriched in HC compared to AR or ARAS with negative or nearly zero log2 Fold Change (FC). Only one pathway, D-galactose degradation I (Leloir pathway; PWY-6317), was enriched (log2 FC>0.75) in the ARAS group in comparison with the control group (Fig. 4B). The enzymes of this pathway catalyze the conversion of D-galactose to D-glucopyranose 6-phosphate, which allows microorganisms to ultimately use D-galactose for glycolysis. Interestingly, the comparison AR versus ARAS yielded no significant results (p-value > 0.1), suggesting both conditions share a similar oral mycobiome functional signature.

3.3. Mycobiome interactions

We also investigated the potential direct or indirect interactions among fungal groups. We inferred inverse covariance networks using the Spiec-Easi model to compare the structure and connectivity of the oral mycobiome. The HC group network was sparsely connected and characterized by positive interactions (8 modules), as evidenced by low degree connectivity (range 1–2) and betweenness centrality (range 0–1) (Fig. 5). Likewise, the ARAS network (9 modules) also exhibited low degree connectivity (range 1–5) but a higher betweenness centrality (range 0–35), suggesting the presence of key nodes connecting taxa. Nodes with highest betweenness centrality belonged to the genera *Conocybe* and *Rhizophlyctis* (14 and 35, respectively). The AR network, in

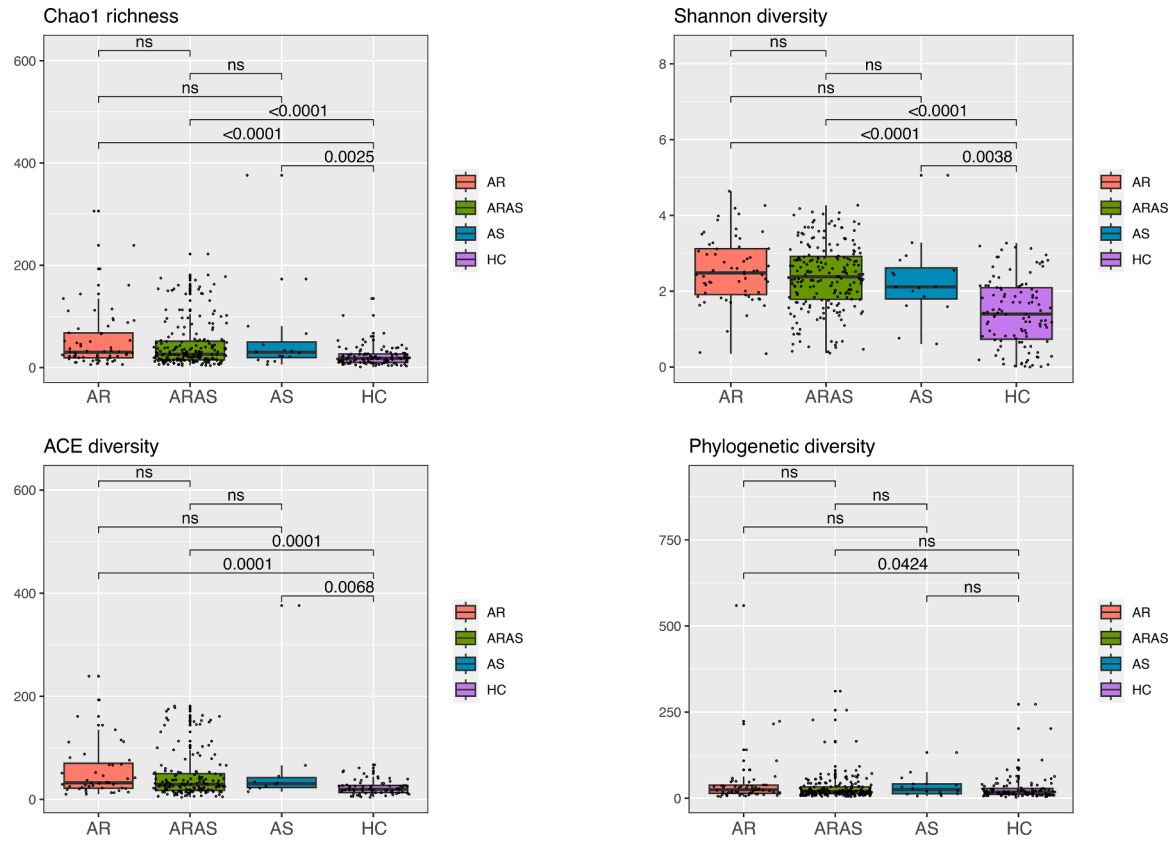

Fig. 2. Alpha-diversity estimates (Chao1, Shannon, ACE, and phylogenetic diversity) and statistical significance (LME model test) in oral fungal communities from participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS), asthma (AS) and healthy controls (HC). ns=not significant.

turn, exhibited moderate degree of connectivity (range 1–4; 17 modules), but a high betweenness centrality (0–350). The top 5 fungal genera with the highest betweenness centrality were *Phlebia, Sidera, Symmetrospora, Skeletocutis*, and *Bullera*, suggesting they play key roles in the structure of the oral mycobiota in patients with allergic rhinitis (Fig. 5). Overall, the structure of the three networks was different, as revealed by their low node and edge overlap. The HC and ARAS networks only shared one node, and the HC and AR networks shared five nodes. No nodes were shared among the two rhinitic or three diseased groups. Similarly, the networks shared no edges, suggesting the structure of the mycobiota in controls and disease groups is profoundly different.

4. Discussion

The contribution of the upper airways mycobiome to allergic rhinitis and asthma is practically unknown (Yuan et al., 2023; Jung et al., 2015). This cross-sectional study compares the oral mycobiome of 349 Portuguese individuals with allergic rhinitis (with and without comorbid asthma), asthma and healthy controls.

The studied oral mycobiomes were comprised of basically two phyla (Ascomycota and Basidiomycota) and 14 genera (Fig. 1 and Table 1). These two phyla and all dominant genera (mean relative proportions ≥3 %) have been previously described in the airways of both healthy, asthmatic and rhinitic individuals, although with different abundances (van Tilburg Bernardes et al., 2020; Carpagnano et al., 2016; Goldman et al., 2018; Rick et al., 2020; Yuan et al., 2023; Jung et al., 2015; Dupuy et al., 2014; Ghannoum et al., 2010). We detected common opportunistic pathogenic fungi like *Malassezia, Aspergillus, Candida* and *Penicillium* (Badiee and Hashemizadeh, 2014). Moreover, *Alternaria* has been associated with AR symptoms (Andersson et al., 2003). This suggests that the mouth may act as a reservoir for opportunistic respiratory pathogens (Dong et al., 2021), which can then enrich nasal–pharyngeal mycobiota (Fan et al., 2020) and lead to respiratory disease.

The mycobiomes of healthy controls differed greatly in composition from those of participants with chronic respiratory illnesses. The oral mycobiome of healthy controls contained 18.2 % unique ASVs, while the AR, ARAS and AS bacteriomes contained 17.3 %, 42.8 % and 4.7 % unique ASVs, respectively (Table S2 and Fig. S1). These ASVs may represent biomarkers of disease for each clinical group. Further genomic studies are needed to confirm these results and their potential as therapeutic targets for rhinitis and asthma (Pérez-Losada et al., 2023a, 2023b; Castro-Nallar et al., 2015; Pérez-Losada et al., 2015).

Ascomycota and Basidiomycota varied significantly ($P < 0.025$) in their mean relative proportions between ARAS or AR and HC (Table 1). A total of five dominant genera also varied significantly ($P < 0.039$) between healthy and allergic rhinitic samples with or without asthma comorbidity (Table 1). None of the other pairwise comparison resulted significantly different. *Cladosporium* and *Rhodotorula* were significantly more abundant in rhinitic patients, while *Aspergillus, Aleurina* and *Candida* increased in healthy controls. No other studies have compared the oral mycobiotas of rhinitic and healthy individuals, but a single study of the nasal mycobiome (Jung et al., 2015) did not discover significant differences between both groups, although showed a higher abundance of Basidiomycota than Ascomycota in rhinitic patients, which disagrees with our findings here. Compositional changes in these fungal taxa may provide insights into the pathobiology of allergic rhinitis. Further studies are needed to confirm our findings and the contribution of fungal dysbiosis to chronic inflammatory disease (Nguyen et al., 2015; van Tilburg Bernardes et al., 2020; Goldman et al., 2018; Rick et al., 2020; Yuan et al., 2023).

Fungal alpha-diversity (species richness and evenness) was significantly ($P < 0.007$) higher in ARAS, AR and AS compared to HC for all indices but PD (Fig. 2), which only resulted significant for AR-HC ($P =$

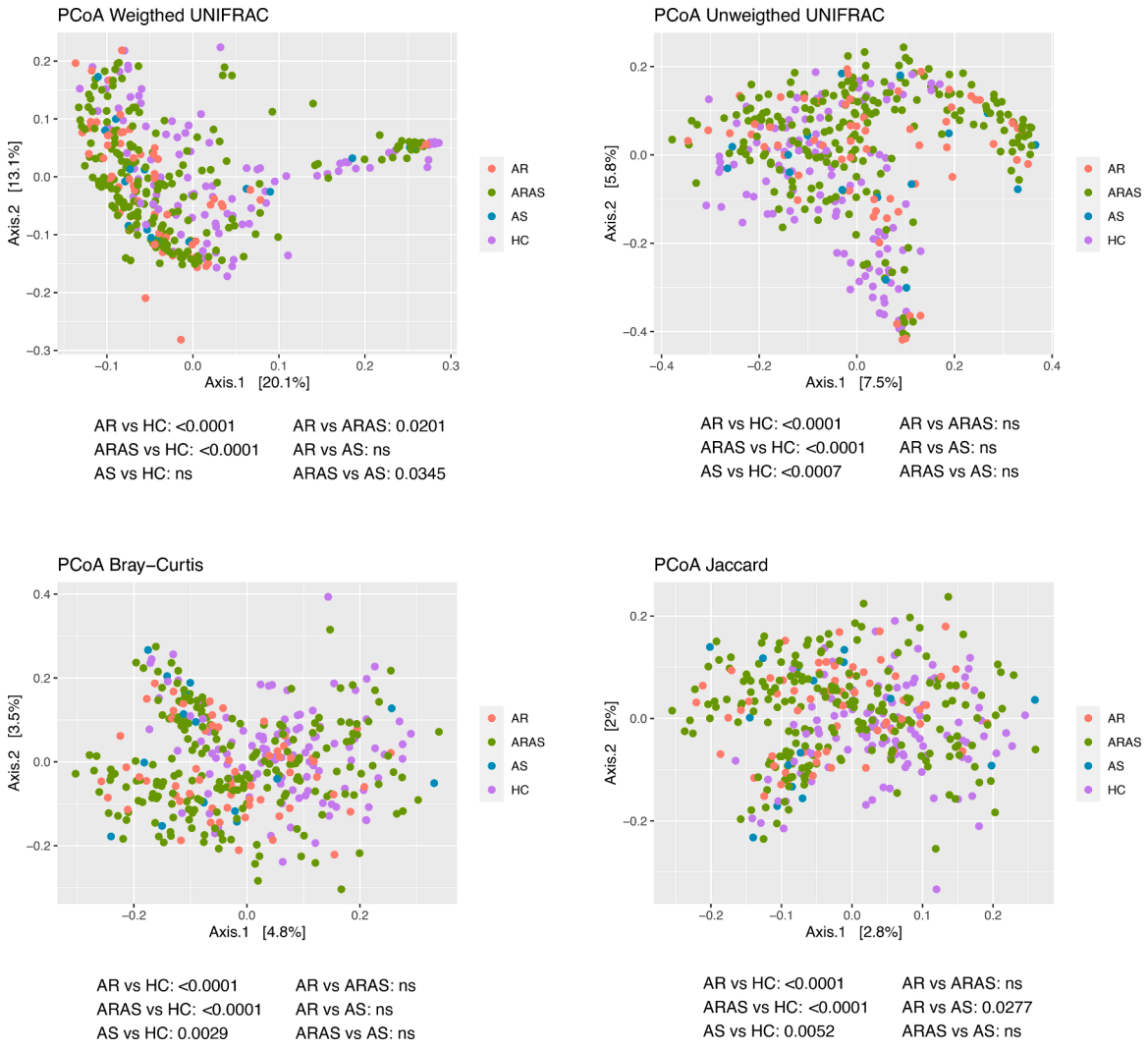

Fig. 3. Principal coordinates analysis (PCoA) plots of beta-diversity estimates (Unifrac, Bray-Curtis and Jaccard indices) and statistical significance (adonis test) in oral fungal communities from participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS), asthma (AS) and healthy controls (HC). ns=not significant.

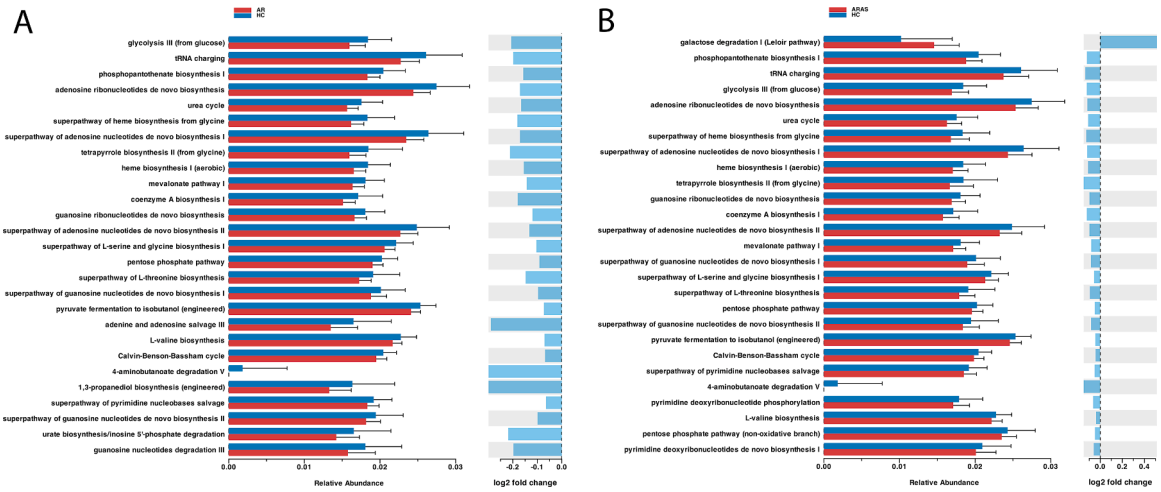

Fig. 4. Differential abundance analysis (Wald's test; adjusted p-value <0.01) of functional profiles in the oral mycobiomes of participants with allergic rhinitis (AR) and healthy controls (HC) (A), and AR participants with comorbid asthma (ARAS) and HC (B).

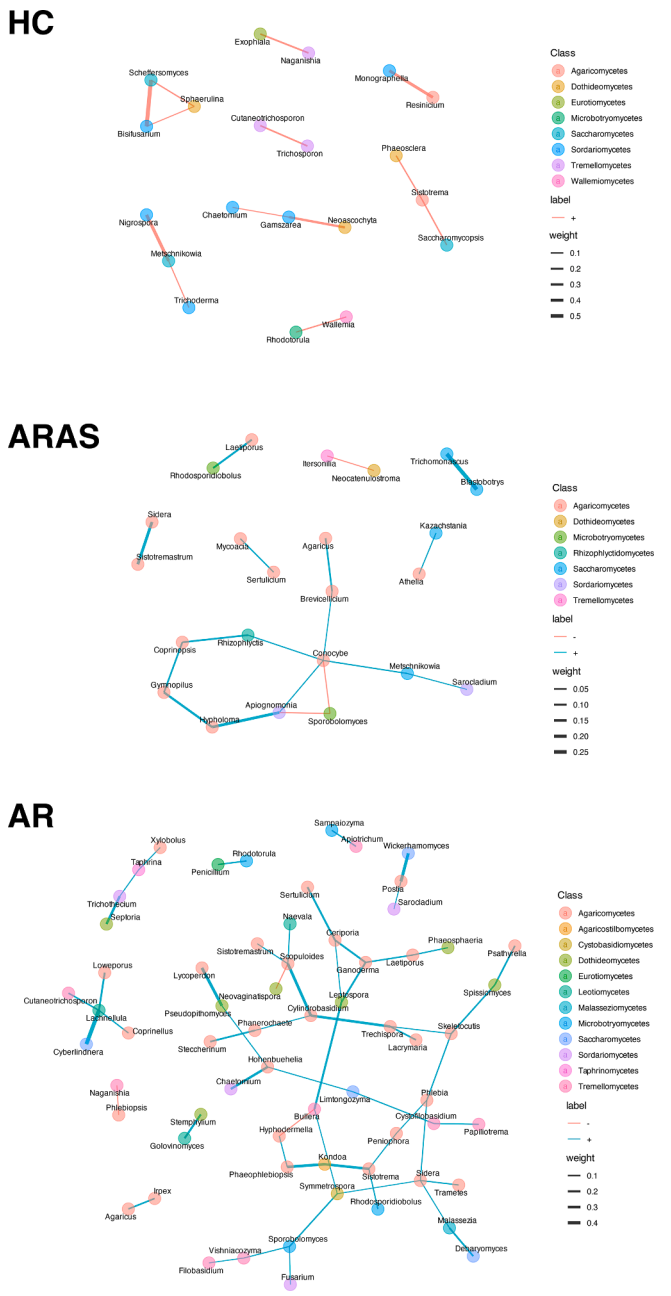

Fig. 5. Spiec-Easi networks of fungal taxa in the oral mycobiomes of participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS) and healthy controls (HC). Nodes represent taxa connected by edges whose width (0.05 to 0.5) is proportional to the strength of their association. Cyan and pink edges indicate positive and negative correlations, respectively.

0.0424). All other pairwise comparisons resulted unsignificant. Again, the single study that has explored the diversity of the upper airway microbiota, albeit the nasal cavity, in individuals with rhinitis (Jung et al., 2015) also reported higher estimates of Shannon diversity for the AR group than for the healthy patients. Another study of the nasal mycobiome during asthma has also shown higher alpha-diversity during asthma exacerbations (Yuan et al., 2023). If confirmed, this may suggest that allergic rhinitis and asthma may increase microbial diversity in the upper airways, as seen in previous studies of the bacteriome (Gan et al., 2021; Kim et al., 2022; Pérez-Losada et al., 2023a, 2023b; Choi et al., 2014).

AR, ARAS and AS shown significant differences in community structure (i.e., beta-diversity) compared to HC for all tests but one involving AS-HC (Fig. 3). Additionally, pairwise comparisons of respiratory disease groups resulted not significant for three of the four indices compared. A previous study of the nasal mycobiota (Jung et al., 2015) also revealed that AR and HC communities were very differentiated. Hence, as indicated before (Pérez-Losada et al., 2023a, 2023b), these results suggest that fungal compositional shifts in the upper airways may be a reliable predictor of allergic rhinitis or asthma in the upper airways, given their lower stochasticity related to dysmycobiosis (Ma, 2020; Ma et al., 2019).

As with the taxonomic diversity and composition of the oral mycobiome of allergic rhinitis, its functional component is largely underexplored. Here, we used an imputation method to indirectly explore the functional potential of the oral mycobiome (Fig. 4). We found modest yet significant differences in metabolic pathway abundance when comparing AR and ARAS to HC. Most of the differences indicated a significant reduction in the relative abundance of metabolic pathways in samples from AR and ARAS groups. These findings are consistent with the taxonomic analyses and suggests that taxonomy follows function in the oral mycobiome, either in health or disease. One exemption was the D-galactose degradation pathway, which was more abundant in ARAS patients than control patients. Up to our knowledge, the impact (if any) of the fungal D-galactose degradation pathway in airway human health has not been investigated, hence the implications of this result remain to be validated.

We used a network approach to explore mycobiome interactions in the oral cavity (Fig. 5). Those methods are generally based on co-occurrence or co-variation of microbes' abundance to infer direct or indirect interactions. Positive interactions might be indicative of syntrophy (i.e., a relationship in which one or both organisms benefit nutritionally from the presence of the other) or commensalism, while negative interactions may indicate competition or predation. In our study, the HC group showed fewer significant interactions, all of which were positive. In contrast, the ARAS and notably the AR group exhibited more diverse relationships with multiple modules with positive and negative interactions among fungal taxa. In oral fungal communities, several studies have focused on cross-kingdom bacteria-fungi interactions and their associations with disease (Du et al., 2022). However, previous research has shown that these patterns of co-abundance and exclusion seem to be stable across body sites in the healthy human microbiome and that its alteration can be indicative of underlying disease processes (Faust et al., 2012). In previous studies of the bacteriome in patients with allergic rhinitis (Pérez-Losada et al., 2023a, 2023b, 2018) or of the mycobiome in asthmatics (Huang et al., 2020; Liu et al., 2020), co-occurrence networks in diseased participants exhibited different interactions than in healthy controls. Our novel analyses of the oral mycobiome in rhinitic patients seem to confirm these results, although with the allergic rhinitis (AR) exhibiting a higher and more diverse mycobiome network. Interestingly, despite of the multiple connections of rhinitis and asthma and the proposed concept of a united airway disease (Compalati et al., 2010), recent omic data (Dizier et al., 2007; Lemonnier et al., 2020) suggests that rhinitis alone and rhinitis with comorbid asthma may represent two distinct diseases with different allergen sensitization and onset (Siroux et al., 2018), severity (Savoure et al., 2023) and treatment response (Sousa-Pinto et al., 2022). Moreover, the hypothesis that these two distinct diseases are possibly modulated by the microbiome has been recently proposed (Bousquet et al., 2023). Further research is needed to explore the role of fungi in chronic inflammation, particularly in allergic individuals.

There is emerging evidence of that the airway mycobiome has a significant impact on clinical outcome of chronic respiratory diseases such as asthma (Nguyen et al., 2015). A few studies have already shown that nasal fungal dysbiosis is associated to asthma (Yuan et al., 2023) and allergic rhinitis (Jung et al., 2015); little is known, however, about the role of the oral cavity mycobiota. We have shown that oral dysmycobiosis (i.e., imbalance in the fungal community) may contribute to allergic rhinitis with or without asthma comorbidity. This finding

warrants further research to elucidate the relationship between the oral mycobiota and airway pathology.

Ethics statement

All individuals participating in this study were enrolled through the ASMAPORT Project (PTDC/SAU-INF/27,953/2017). This study was revised and approved by the Comissão de Ética para a Saúde of the Centro Hospitalar Universitário São João / Faculdade de Medicina (Porto, Portugal) in March 2017 (Parecer_58–17). All methods were performed in accordance with the relevant guidelines and regulations. We obtained written consent from all independent participants or their legal guardians using the informed consent documents approved by the Comissão de Ética.

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Supplementary Fig. 1. UpSet plots of amplicon sequence variants (ASVs) in the oral mycobiome of participants with allergic rhinitis (AR), AR with comorbid asthma (ARAS), asthma (AS) and healthy controls (HC).

CRediT authorship contribution statement

Marcos Pérez-Losada: Conceptualization, Methodology, Data curation, Investigation, Validation, Writing – review & editing, Resources, Funding acquisition, Supervision, Project administration, Writing – original draft, Visualization, Formal analysis. **Eduardo Castro-Nallar:** Methodology, Software, Writing – original draft, Visualization, Writing – review & editing, Formal analysis. **Jenaro García-Huidobro:** Methodology, Data curation, Validation, Writing – review & editing. **José Laerte Boechat:** Methodology, Data curation, Validation, Writing – review & editing. **Luis Delgado:** Conceptualization, Methodology, Data curation, Investigation, Validation, Writing – review & editing, Resources, Funding acquisition. **Tiago Azenha Rama:** Methodology, Data curation, Validation, Writing – review & editing. **Manuela Oliveira:** Conceptualization, Methodology, Data curation, Investigation, Validation, Writing – review & editing, Resources, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marcos Pérez Losada reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2024.100300.

Data availability

ITS files, clinical metadata and BioSample attributes for all samples in this study have been deposited in the NCBI (PRJNA1107919).

References

Acevedo-Prado, A., Seoane-Pillado, T., Lopez-Silvarrey-Varela, A., et al., 2022. Association of rhinitis with asthma prevalence and severity. Sci. Rep. 12, 6389.
Andersson, M., Downs, S., Mitakakis, T., Leuppi, J., Marks, G., 2003. Natural exposure to Alternaria spores induces allergic rhinitis symptoms in sensitized children. Pediatr. Allergy Immunol. 14, 100–105.
Azevedo, A.C., Hilario, S., Goncalves, M.F.M., 2023. Microbiome in nasal mucosa of children and adolescents with allergic rhinitis: a systematic review. Children (Basel) 10, 226.
Badiee, P., Hashemizadeh, Z., 2014. Opportunistic invasive fungal infections: diagnosis & clinical management. Indian J. Med. Res. 139, 195–204.
Bandara, H., Panduwawala, C.P., Samaranayake, L.P., 2019. Biodiversity of the human oral mycobiome in health and disease. Oral Dis. 25, 363–371.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
Bender, M.E., Read, T.D., Edwards, T.S., et al., 2020. A comparison of the bacterial nasal microbiome in allergic rhinitis patients before and after immunotherapy. Laryngoscope 130, E882–E888.
Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B (Methodological) 57, 289–300.
Bergeron, C., Hamid, Q., 2005. Relationship between asthma and rhinitis: epidemiologic, pathophysiologic, and therapeutic aspects. Allergy Asthma Clin. Immunol. 1, 81–87.
Bogaert, D., Keijser, B., Huse, S., et al., 2011. Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. PloS One 6, e17035.
Bousquet, P.J., Burbach, G., Heinzerling, L.M., et al., 2009. GA2LEN skin test study III: minimum battery of test inhalent allergens needed in epidemiological studies in patients. Allergy 64, 1656–1662.
Bousquet, J., Hellings, P.W., Agache, I., et al., 2019. Allergic Rhinitis and its Impact on Asthma (ARIA) phase 4 (2018): change management in allergic rhinitis and asthma multimorbidity using mobile technology. J. Allergy Clin. Immunol. 143, 864–879.
Bousquet, J., Melen, E., Haahtela, T., et al., 2023. Rhinitis associated with asthma is distinct from rhinitis alone: the ARIA-MeDALL hypothesis. Allergy 78, 1169–1203.
Brar, T., Nagaraj, S., Mohapatra, S., 2012. Microbes and asthma: the missing cellular and molecular links. Curr. Opin. Pulm. Med. 18, 14–22.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
Carpagnano, G.E., Malerba, M., Lacedonia, D., et al., 2016. Analysis of the fungal microbiome in exhaled breath condensate of patients with asthma. Allergy Asthma Proc. 37, 41–46 the official journal of regional and state allergy societies.
Caspi, R., Billington, R., Keseler, I.M., et al., 2020. The MetaCyc database of metabolic pathways and enzymes - a 2019 update. Nucl. Acids Res. 48, D445–D453.
Castro-Nallar, E., Shen, Y., Freishtat, R.J., et al., 2015. Integrating metagenomics and host gene expression to characterize asthma-associated microbial communities. BMC Med. Genomics 8, 50.
Chen, M., He, S., Miles, P., et al., 2022. Nasal bacterial microbiome differs between healthy controls and those with asthma and allergic rhinitis. Front. Cell Infect. Microbiol. 12, 841995.
Choi, C.H., Poroyko, V., Watanabe, S., et al., 2014. Seasonal allergic rhinitis affects sinonasal microbiota. Am. J. Rhinol. Allergy 28, 281–286.
Compalati, E., Ridolo, E., Passalacqua, G., Braido, F., Villa, E., Canonica, G.W., 2010. The link between allergic rhinitis and asthma: the united airways disease. Expert. Rev. Clin. Immunol. 6, 413–423.
Cook, R.D., 1977. Detection of influential observation in linear regression. Technometrics 19, 15–18.
de Dios Caballero, J., Canton, R., Ponce-Alonso, M., et al., 2022. The human mycobiome in chronic respiratory diseases: current situation and future perspectives. Microorganisms 10, 810.
Dharmage, S.C., Perret, J.L., Custovic, A., 2019. Epidemiology of asthma in children and adults. Front. Pediatr. 7, 246.
Diaz, P.I., 2021. Dongari-Bagtzoglou A: critically appraising the significance of the oral mycobiome. J. Dent. Res. 100, 133–140.
Dickson, R.P., Huffnagle, G.B., 2015. The lung microbiome: new principles for respiratory bacteriology in health and disease. PLoS Pathog. 11, e1004923.
Dinwiddie, D.L., Denson, J.L., Kennedy, J.L., 2018. Role of the airway microbiome in respiratory infections and asthma in children. Pediatr. Allergy Immunol. Pulmonol. 31, 236–240.
Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.

Dizier, M.H., Bouzigon, E., Guilloud-Bataille, M., et al., 2007. Evidence for a locus in 1p31 region specifically linked to the co-morbidity of asthma and allergic rhinitis in the EGEA study. Hum. Hered. 63, 162–167.
Dong, J., Li, W., Wang, Q., et al., 2021. Relationships between oral microecosystem and respiratory diseases. Front. Mol. Biosci. 8, 718222.
Douglas, G.M., Maffei, V.J., Zaneveld, J.R., et al., 2020. PICRUSt2 for prediction of metagenome functions. Nat. Biotechnol. 38, 685–688.
Du, Q., Ren, B., Zhou, X., Zhang, L., Xu, X., 2022. Cross-kingdom interaction between Candida albicans and oral bacteria. Front. Microbiol. 13, 911623.
Dupuy, A.K., David, M.S., Li, L., et al., 2014. Redefining the human oral mycobiome with improved practices in amplicon-based taxonomy: discovery of Malassezia as a prominent commensal. PloS One 9, e90899.
Falcão, H., Ramos, E., Marques, A., Barros, H., 2008. Prevalence of asthma and rhinitis in 13 year old adolescents in Porto. Portugal. Rev. Port. Pneumol. 14, 747–768.
Fan, C., Guo, L., Gu, H., Huo, Y., Lin, H., 2020. Alterations in oral-nasal-pharyngeal microbiota and salivary proteins in mouth-breathing children. Front. Microbiol. 11, 575550.
Faust, K., Sathirapongsasuti, J.F., Izard, J., et al., 2012. Microbial co-occurrence relationships in the human microbiome. Plos Comput. Biol. 8, e1002606.
Ferreira-Magalhaes, M., Pereira, A.M., Sa-Sousa, A., et al., 2015. Asthma control in children is associated with nasal symptoms, obesity, and health insurance: a nationwide survey. Pediatr. Allergy Immunol. 26, 466–473 official publication of the European Society of Pediatric Allergy and Immunology.
Ferreira-Magalhaes, M., Sa-Sousa, A., Morais-Almeida, M., et al., 2016. Asthma-like symptoms, diagnostic tests, and asthma medication use in children and adolescents: a population-based nationwide survey. J. Asthma 53, 269–276 official journal of the Association for the Care of Asthma.
Fonseca, J., Taveira-Gomes, T., Pereira, A.M., et al., 2021. [ARIA 2019: an integrated care pathway for allergic rhinitis in Portugal]. Acta Med. Port. 34, 144–157.
Frati, F., Salvatori, C., Incorvaia, C., et al., 2018. The role of the microbiome in asthma: the gut(-)lung axis. Int. J. Mol. Sci. 20, 123.
Gan, W., Yang, F., Meng, J., Liu, F., Liu, S., Xian, J., 2021. Comparing the nasal bacterial microbiome diversity of allergic rhinitis, chronic rhinosinusitis and control subjects. Eur. Arch. Otorhinolaryngol. 278, 711–718.
Ghannoum, M.A., Jurevic, R.J., Mukherjee, P.K., et al., 2010. Characterization of the oral fungal microbiome (mycobiome) in healthy individuals. PLoS Pathog. 6, e1000713.
Goldman, D.L., Chen, Z., Shankar, V., Tyberg, M., Vicencio, A., Burk, R., 2018. Lower airway microbiota and mycobiota in children with severe asthma. J. Allergy Clin. Immunol. 141, 808–811 e807.
Huang, Y.J., Boushey, H.A., 2014. The microbiome and asthma. Ann. Am. Thorac. Soc. 11 (Suppl 1), S48–S51.
Huang, Y.J., Boushey, H.A., 2015. The microbiome in asthma. J. Allergy Clin. Immunol. 135, 25–30.
Huang, C., Yu, Y., Du, W., et al., 2020. Fungal and bacterial microbiome dysbiosis and imbalance of trans-kingdom network in asthma. Clin. Transl. Allergy 10, 42.
Hufnagl, K., Pali-Scholl, I., Roth-Walter, F., Jensen-Jarolim, E., 2020. Dysbiosis of the gut and lung microbiome has a role in asthma. Semin. Immunopathol. 42, 75–93.
Jung, W.H., Croll, D., Cho, J.H., Kim, Y.R., Lee, Y.W., 2015. Analysis of the nasal vestibule mycobiome in patients with allergic rhinitis. Mycoses 58, 167–172.
Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.
Kim, H., Bouchard, J., Renzi, P.M., 2008. The link between allergic rhinitis and asthma: a role for antileukotrienes? Can. Respir. J. 15, 91–98.
Kim, H.J., Kim, J.H., Han, S., Kim, W., 2022. Compositional alteration of the nasal microbiome and Staphylococcus aureus-characterized dysbiosis in the nasal mucosa of patients with allergic rhinitis. Clin. Exp. Otorhinolaryngol. 15, 335–345.
Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120.
Kurtz, Z.D., Muller, C.L., Miraldi, E.R., Littman, D.R., Blaser, M.J., Bonneau, R.A., 2015. Sparse and compositionally robust inference of microbial ecological networks. Plos Comput. Biol. 11, e1004226.
Lal, D., Keim, P., Delisle, J., et al., 2017. Mapping and comparing bacterial microbiota in the sinonasal cavity of healthy, allergic rhinitis, and chronic rhinosinusitis subjects. Int. Forum Allergy Rhinol. 7, 561–569.
Lemonnier, N., Melen, E., Jiang, Y., et al., 2020. A novel whole blood gene expression signature for asthma, dermatitis, and rhinitis multimorbidity in children and adolescents. Allergy 75, 3248–3260.
Li, R., Li, J., Zhou, X., 2024. Lung microbiome: new insights into the pathogenesis of respiratory diseases. Signal Transd. Target. Therapy 9, 19.
Licari, A., Brambilla, I., Marseglia, A., De Filippo, M., Paganelli, V., Marseglia, G.L., 2018. Difficult vs. severe asthma: definition and limits of asthma control in the pediatric population. Front. Pediatr. 6, 170.
Liu, H.Y., Li, C.X., Liang, Z.Y., et al., 2020. The interactions of airway bacterial and fungal communities in clinically stable asthma. Front. Microbiol. 11, 1647.
Liu, C., Cui, Y., Li, X., Yao, M., 2021. microeco: an R package for data mining in microbial community ecology. FEMS Microbiol. Ecol. 97, fiaa255.
Losol, P., Choi, J.P., Kim, S.H., Chang, Y.S., 2021. The role of upper airway microbiome in the development of adult asthma. Immune Netw. 21, e19.
Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550.
Ma, Z.S., Li, L., Gotelli, N.J., 2019. Diversity-disease relationships and shared species analyses for human microbiome-associated diseases. ISME J. 13, 1911–1919.
Ma, Z.S., 2020. Testing the Anna Karenina principle in human microbiome-associated diseases. iScience 23, 101007.
Man, W.H., de Steenhuijsen Piters, W.A., Bogaert, D., 2017. The microbiota of the respiratory tract: gatekeeper to respiratory health. Nat. Rev. Microbiol. 15, 259–270.
McMurdie, P.J., Holmes, S., 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217.
McMurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10, e1003531.
Mims, J.W., 2015. Asthma: definitions and pathophysiology. Int. Forum Allergy Rhinol. 5,, 1: S2-6.
Muc, M., Mota-Pinto, A., Padez, C., 2014. Prevalence of asthma and rhinitis symptoms among children living in Coimbra. Portugal. Rev. Port. Pneumol. 20, 208–210.
Nguyen, L.D., Viscogliosi, E., Delhaes, L., 2015. The lung mycobiome: an emerging field of the human respiratory microbiome. Front. Microbiol. 6, 89.
Nilsson, R.H., Larsson, K.H., Taylor, A.F.S., et al., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucl. Acids Res. 47, D259–D264.
Oliveira, M., Oliveira, D., Lisboa, C., Boechat, J.L., Delgado, L., 2023. Clinical manifestations of human exposure to fungi. J. Fungi (Basel) 9, 381.
Pawankar, R., 2006. Allergic rhinitis and asthma: are they manifestations of one syndrome? Clin. Exp. Allergy 36, 1–4 journal of the British Society for Allergy and Clinical Immunology.
Pereira, C., Valero, A., Loureiro, C., et al., 2006. Iberian study of aeroallergens sensitisation in allergic rhinitis. Eur. Ann. Allergy Clin. Immunol. 38, 186–194.
Pérez-Losada, M., Castro-Nallar, E., Laerte Boechat, J., et al., 2023a. The oral bacteriomes of patients with allergic rhinitis and asthma differ from that of healthy controls. Front. Microbiol. 14, 1197135.
Pérez-Losada, M., Castro-Nallar, E., Laerte Boechat, J., et al., 2023b. Nasal bacteriomes of patients with asthma and allergic rhinitis show unique composition, structure, function and interactions. Microorganisms 11, 683.
Pite, H., Pereira, A.M., Morais-Almeida, M., Nunes, C., Bousquet, J., Fonseca, J.A., 2014. Prevalence of asthma and its association with rhinitis in the elderly. Respir. Med. 108, 1117–1126.
Price, M.N., Dehal, P.S., Arkin, A.P., 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PloS One 5, e9490.
Pérez-Losada, M., Castro-Nallar, E., Bendall, M.L., Freishtat, R.J., Crandall, K.A., 2015. Dual transcriptomic profiling of host and microbiota during health and disease in pediatric asthma. PloS One 10, e0131819.
Pérez-Losada, M., Crandall, K.A., Freishtat, R.J., 2016a. Comparison of two commercial DNA extraction kits for the analysis of nasopharyngeal bacterial communities. AIMS Microbiol. 2, 108–119.
Pérez-Losada, M., Crandall, K.A., Freishtat, R.J., 2016b. Two sampling methods yield distinct microbial signatures in the nasopharynges of asthmatic children. Microbiome 4, 25.
Pérez-Losada, M., Alamri, L., Crandall, K.A., Freishtat, R.J., 2017. Nasopharyngeal microbiome diversity changes over time in children with asthma. PloS One 12, e0170543.
Pérez-Losada, M., Authelet, K.J., Hoptay, C.E., Kwak, C., Crandall, K.A., Freishtat, R.J., 2018. Pediatric asthma comprises different phenotypic clusters with unique nasal microbiotas. Microbiome 6, 179.
Quast, C., Pruesse, E., Yilmaz, P., et al., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41, D590–D596.
Raita, Y., Pérez-Losada, M., Freishtat, R.J., et al., 2021. Integrated omics endotyping of infants with respiratory syncytial virus bronchiolitis and risk of childhood asthma. Nat. Commun. 12, 3601.
Rick, E.M., Woolnough, K.F., Seear, P.J., et al., 2020. The airway fungal microbiome in asthma. Clin. Exp. Allergy 50, 1325–1341 journal of the British Society for Allergy and Clinical Immunology.
RStudio RT, 2015. Integrated Development For R. RStudio. IncBoston, MA.
Sa-Sousa, A., Morais-Almeida, M., Azevedo, L.F., et al., 2012. Prevalence of asthma in Portugal - the Portuguese national asthma survey. Clin. Transl. Allergy 2, 15.
Savoure, M., Bousquet, J., Jaakkola, J.J.K., Jaakkola, M.S., Jacquemin, B., Nadif, R., 2022. Worldwide prevalence of rhinitis in adults: a review of definitions and temporal evolution. Clin. Transl. Allergy 12, e12130.
Savoure, M., Bousquet, J., Leynaert, B., et al., 2023. Rhinitis phenotypes and multimorbidities in the general population: the CONSTANCES cohort. Eur. Respir. J. 61, 2200943.
Silva, D., Severo, M., Paciencia, I., et al., 2019. Setting definitions of childhood asthma in epidemiologic studies. Pediatr. Allergy Immunol. 30, 708–715 official publication of the European Society of Pediatric Allergy and Immunology.
Siroux, V., Ballardini, N., Soler, M., et al., 2018. The asthma-rhinitis multimorbidity is associated with IgE polysensitization in adolescents and adults. Allergy 73, 1447–1458.
Small, P., Keith, P.K., Kim, H., 2018. Allergic rhinitis. Allergy Asthma Clin. Immunol. 14, 51.
Sousa-Pinto, B., Schunemann, H.J., Sa-Sousa, A., et al., 2022. Comparison of rhinitis treatments using MASK-air(R) data and considering the minimal important difference. Allergy 77, 3002–3014.
Steelant, B., Farre, R., Wawrzyniak, P., et al., 2016. Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression. J. Allergy Clin. Immunol. 137, 1043–1053 e1045.
Steelant, B., Seys, S.F., Van Gerven, L., et al., 2018. Histamine and T helper cytokine-driven epithelial barrier dysfunction in allergic rhinitis. J. Allergy Clin. Immunol. 141, 951–963 e958.
Team RDC, 2008. R: A Language and Environment For Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

Teo, S.M., Mok, D., Pham, K., et al., 2015. The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. Cell Host Microbe 17, 704–715.

Thompson, L.R., Sanders, J.G., McDonald, D., et al., 2017. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 551, 457–463.

Todo-Bom, A., Loureiro, C., Almeida, M.M., et al., 2007. Epidemiology of rhinitis in Portugal: evaluation of the intermittent and the persistent types. Allergy 62, 1038–1043.

Valero, A., Pereira, C., Loureiro, C., et al., 2009. Interrelationship between skin sensitization, rhinitis, and asthma in patients with allergic rhinitis: a study of Spain and Portugal. J. Investig. Allergol. Clin. Immunol. 19, 167–172.

van Tilburg Bernardes, E., Gutierrez, M.W., Arrieta, M.C., 2020. The fungal microbiome and asthma. Front. Cell Infect. Microbiol. 10, 583418.

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.

Yang, C., Mai, J., Cao, X., Burberry, A., Cominelli, F., Zhang, L., 2023. ggpicrust2: An R package for PICRUSt2 predicted functional profile analysis and visualization. Bioinformatics 39, btad470.

Yuan, H., Liu, Z., Dong, J., et al., 2023. The fungal microbiome of the upper airway is associated with future loss of asthma control and exacerbation among children with asthma. Chest. 164, 302–313.