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The Gut Microbiome of Adults with Allergic Rhinitis Is Characterised by Reduced Diversity and an Altered Abundance of Key Microbial Taxa Compared to Controls

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Microbiome · Allergic rhinitis · 16s rRNA gene · Species richness · Gastrointestinal

Abstract
Introduction: Unique gut microbial colonisation patterns are associated with the onset of allergic disease in infants; however, there is insufficient evidence to determine if aberrant microbial composition patterns persist in adult allergic rhinitis (AR) sufferers. ***Objective:*** To compare the gut microbiome composition between adult AR sufferers and controls. ***Methods:*** Gut microbial composition in stool samples was compared between 57 adult AR sufferers (39.06 ± 13.29 years) and 23 controls (CG; 36.55 ± 10.51 years) via next-generation sequencing of the V3–V4 hypervariable regions of the 16S rRNA gene. Taxonomic classification and identity assignment was performed using a reference-based approach with the NCBI database of 16S rRNA gene sequences. ***Results:*** Species richness determined via the Shannon index was significantly reduced in the AR cohort compared to the CG (4.35 ± 0.59 in AR vs. 4.65 ± 0.55 in CG, $p = 0.037$); trends for reductions in operational taxonomic unit (OTU) counts, inverse Simpson, and CHAO1 diversity indices were also noted. Bacteroidetes ($p = 0.014$) was significantly more abundant in the AR group than in the CG. In contrast, the Firmicutes phylum was significantly less abundant in the AR group than in the CG ($p = 0.006$). An increased abundance of *Parabacteroides* ($p = 0.008$) and a reduced abundance of *Oxalobacter* ($p = 0.001$) and Clostridiales ($p = 0.005$) were also observed in the AR cohort compared to the CG. ***Conclusion:*** Adult AR sufferers have a distinct gut microbiome profile, marked by a reduced microbial diversity and altered abundance of certain microbes compared to controls. The results of this study provide evidence that unique gut microbial patterns occur in AR sufferers in adulthood and warrant further examination in the form of mechanistic studies.

Introduction

The gastrointestinal microbiota plays an important role in the development and regulation of local and systemic immunity. Indeed, several immune-mediated conditions [1–7], including allergic disease [8–11], have been

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associated with abnormal gut microbiome composition. Allergic rhinitis (AR) is the most prevalent allergic disease and is characterised by a T helper (Th) 2 polarised response, which promotes IgE-mediated inflammation in the nasal mucosa following allergen exposure. Polarisation of the Th2 phenotype develops in utero and persists in neonates at birth [12, 13]. Immune homeostasis and maturation towards a Th1 phenotype is dependent on colonisation of the gut with commensal microbes [14]. Data generated from germ-free mouse models have shown that commensal gut microbes regulate Th2 responses [15], with recent evidence suggesting this occurs via induction of enteric Th17 and T regulatory cells [16].

The relationship between the gut microbial composition and the onset of allergic disease has been extensively studied in paediatric populations and has been reviewed elsewhere [11]. A consistent finding in these paediatric studies is that lower microbial diversity occurs in the intestine of allergic infants than in non-allergic infants. While differences in microbial composition between allergic and non-allergic infants have been reported, a consensus of an "allergic microbiome profile" cannot be reached. Heterogeneity in study design features, including allergic disease classification, age of subjects, and microbiome identification tools used, complicate our understanding of the microbiome profile of allergic infants. Furthermore, the microbiome profile of allergic infants has been shown to differ by time of sample collection, where samples collected before the onset of allergic disease differ from samples collected during active disease. These data suggest that there is a time- or age-related effect on the development of gut microbiome, relative to atopy.

The gut microbiome is known to undergo rapid increases in bacterial diversity up to approximately 3 years of age [17]. As such, it remains unclear if findings from paediatric studies extend to adult populations. Only a limited number of studies have examined the microbiota of adults with existing allergic disease. Of these few studies conducted, reduced total count of bacteria and anaerobic bacteria was reported in adult atopic dermatitis patients (n = 11) compared to the healthy controls (n = 14) [18]. This study employed bacterial culture techniques to count and identify specific bacterial taxa and its drawbacks including exclusion of "nonculturable" bacteria are well-recognised. 16s rRNA gene-based sequencing has been recently used to circumvent these restrictions, whereby a greater abundance of genera *Faecalibacterium* and *Bifidobacterium* is reported to exist in the faecal microbiota of adult allergic asthma sufferers (n = 21) compared to healthy individuals (n = 22) [8]. Similar microbial identification tools were used in the large-scale "American Gut Project," which comprised 1,879 participants. The results of this trial revealed a decreased microbial diversity in participants with self-reported drug, food, and seasonal allergies, a decreased abundance of Clostridiales, and a higher abundance of Bacteroidales in participants with self-reported seasonal allergies [10]. Given that allergy subtypes are mediated by different inflammatory pathways [19] and perhaps relate to different gut microbial profiles, there may be merit in classifying subjects by allergy phenotypes rather than self-reported allergen sensitivity.

To the authors' knowledge, no faecal microbiota studies employing next-generation sequencing technology have been conducted in adult AR sufferers. This study investigated this gap in knowledge by comparing the gut microbial composition between adults with well-defined AR and controls without AR.

Materials and Methods

Study Design

This study was designed as a cross-sectional study to characterise differences in the gut microbiota between adults with established AR (n = 57) and adults with no history of AR (n = 23; control group). All participants attended appointments at the Queensland Allergy Services Clinic in Southport (Gold Coast, Australia) and the Clinical Trial Unit at Griffith University (Gold Coast, Australia) for allergy testing and collection of blood and stool samples. This study was approved by the Griffith University Human Research Ethics Committee (Approval No. 2015/564/HREC; 2016/279).

Participant Selection

Men and women aged 18–65 years were recruited to the study. The age of participants was closely monitored throughout the recruitment process, and targeted age recruitment of participants was undertaken (when necessary) to ensure that the age profile of the AR and non-AR group was similar. Adults with established AR included both seasonal and perennial AR sufferers with a greater than 2-year history of AR symptoms and a positive allergic response to dust mites or grass pollens. Allergy status was confirmed with a skin prick test against a panel of dust mite and plant pollen allergens, as described previously [20, 21]. AR participants were symptomatic at the time of sample collection and symptom severity was determined using the validated mini Rhinoconjunctivitis Quality of Life Questionnaire (mRQLQ) consisting of 14 questions separated into 5 domains: activities, practical problems, nose symptoms, eye symptoms, and other symptoms [22]. All items on the questionnaire were rated on a 7-point Likert scale (0–6), with each item averaged to give a maximum overall score of 6.

AR subjects were excluded from participating if they suffered from non-AR (vasomotor rhinitis), consumed probiotics in the previous 8–12 weeks, were treated with oral corticosteroids within

Table 1. Demographic and clinical features of AR participants and CG

	All mean	AR mean ± SD	CG mean ± SD	*p* value
N	80	57	23	–
Age, years	38.34±12.54	39.06±13.29	36.55±10.51	0.421
Sex, M/F	32/48 (60% F)	22/35 (61% F)	10/13 (57% F)	0.687
Height, cm	172.16±9.83	171.07±9.80	174.85±9.60	0.120
Weight, kg	75.90±16.20	76.27±14.95	75.00±19.31	0.779
BMI, kg/m^2	25.47±4.28	25.96±4.08	24.25±4.61	0.105
Ethnicity (% Caucasian)	87.50	84.21	95.65	0.161
Immune measures				
WBC count (×10^9/L)	6.48±1.69	6.82±1.72	5.63±1.30	0.004
Lymphocytes (×10^9/L)	2.09±0.66	2.21±0.66	1.81±0.59	0.014
Eosinophils (×10^9/L)	0.30±0.26	0.37±0.27	0.11±0.08	<0.00001
Neutrophils (×10^9/L)	3.52±1.16	3.64±1.21	3.22±0.98	0.136
Basophils (×10^9/L)	0.05±0.04	0.05±0.04	0.04±0.03	0.110
ESR, mm/h	9.10±9.83	10.12±10.70	6.57±6.81	0.144
Disease characteristics				
Co-allergy to dust mites and pollen, %	42.5	59.65	0	–
Dust mite only, %	23.75	33.33	0	–
Pollen only, %	5	7.02	0	–

AR, allergic rhinitis; CG, control group; M, male; F, female; ESR, erythrocyte sedimentation rate.

the previous 6 months or antibiotics within the previous 30 days, used anti-inflammatory or immune-modulating medications, had existing respiratory disease including asthma, nasal polyposis or chronic obstructive pulmonary disorder, had existing immune dysfunction (other than allergies) or gastrointestinal tract diseases or disorders, were ill or had infectious disease at the time of enrolment, or were pregnant at the time of enrolment.

Individuals were recruited to the study as controls (CG) if they reported no history of AR, tested negative to the panel of dust mite and plant pollens, and were free from chronic disease. Participants were excluded from the study if they consumed probiotics in the previous 8–12 weeks, had taken antibiotics within the previous 30 days, used anti-inflammatory or immune-modulating medications, had existing respiratory disease, immune dysfunction or gastrointestinal disease or disorder, were ill or had an infectious disease at the time of enrolment, or were pregnant at the time of enrolment.

Blood Sample Collection

Venous blood samples were collected for analysis of full blood count including white cell differential (QML Pathology, Murarrie, QLD, Australia). In addition, erythrocyte sedimentation rate (ESR) over 1 h was measured using fresh blood samples collected in sodium citrate tubes and using commercially available Vacuette ESR pipettes (Greiner Bio-One, Kremsmünster, Austria) as per the Westergren method [23].

Stool Sample Collection

Subjects were provided with a sample collection kit and instructed to collect a stool sample within 24 h prior to their scheduled study visit. Collection instructions included not to contaminate the sample with urine or water and to store the sample at room temperature until their study visit. Stool samples were frozen at −80°C upon receipt until processing.

Faecal Microbial Composition

DNA was extracted from defrosted stool samples using the method described by Yu et al. [24], which included homogenisation, a combination of chemical and mechanical lysis (using silica/zirconia beads; Daintree Scientific, St Helens, TAS, Australia), salt/alcohol precipitation, and purification using a Qiagen DNAeasy kit (Qiagen, Hilden, Germany). The quality and quantity of DNA were assessed with the NanoDrop 1000 UV-Vis spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Isolated DNA was amplified using universal primers for the V3–V4 region of the microbial 16s rRNA gene (F: 5′-CCTACGGGNGGCWGCAG-3′; R: 5′GACTACHVGGGTATCTAATCC-3′), as described previously [25], and PCR products were sequenced on an Illumina MiSeq system (Illumina, CA, USA) by a commercial provider (Macrogen, Seoul, Korea). Sequence data were processed with CD-HIT-OTU [26] to filter out erroneous and chimaeric reads. Taxonomic classification and identity assignment was performed using a reference-based approach with the NCBI database of 16s rRNA gene sequences.

Statistics

Differences in demographic and clinical measures between groups were assessed with an independent *t* test for continuous variables and a χ^2 test for categorical variables. A one-way ANOVA or an independent *t* test was used to perform the differential α-diversity. Welch's *t* test/unequal variances *t* test was used for heteroscedastic data. A non-parametric Mann-Whitney *U* test was

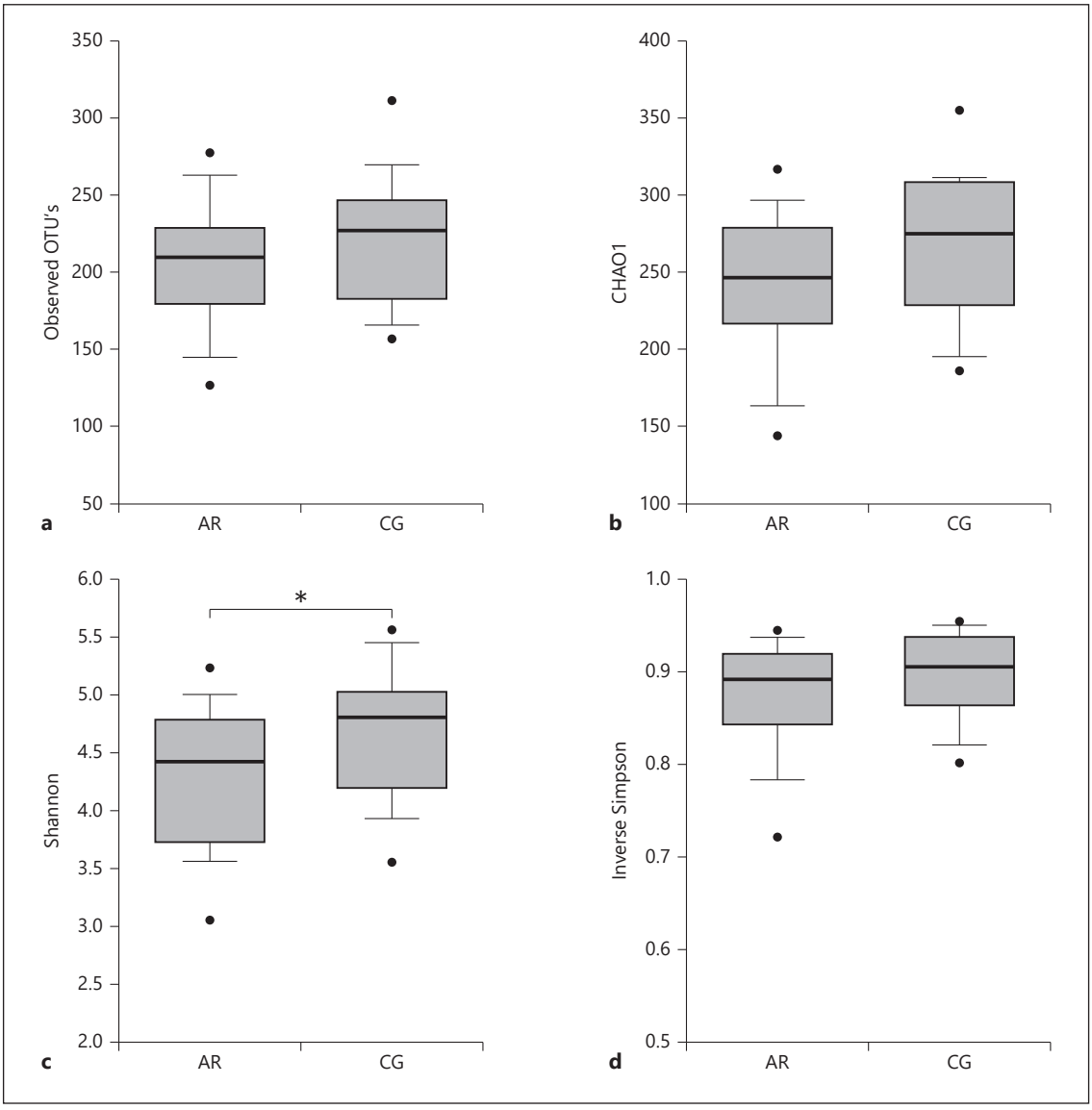


Fig. 1. a–d Alpha diversity metrics for allergic rhinitis (AR) and control group (CG). 16s rRNA sequencing was performed using stool samples collected from AR and CG participants and alpha diversity metrics were determined. **a** The total number of observed operational taxonomic units (OTUs) was lower in the AR group. **b** The CHAO1 index was lower in the AR group. **c** The Shannon index was significantly lower in the AR group (*$p < 0.05$). **d** The inverse Simpson index was lower in the AR group. Outer limits of each box represent the 25th and 75th percentiles, with the median shown as the line within the box. Whiskers (error bars) show the 5th and 95th percentiles, with filled circles showing the outliers.

used to perform the abundance analyses from phylum to species levels. The analysis was confined to taxa with a relative abundance (detected) of >0% and prevalence of >50% in either group (AR or CG). Differences in detection rate of taxa (i.e., detected, >0%; or not detected, 0%; in a given sample) between groups were identified with a χ^2 test. Statistical significance was accepted at $p < 0.05$. Partial least squares-discriminant analysis (PLS-DA) implemented in mixOmics R package [27] was employed for the multivariate analysis. Taxa with a relative abundance of >0% and prevalence <50% in both groups (AR and CG) were excluded from the PLS-DA.

Results

The demographic and clinical characteristics of the cohorts are included in Table 1. The groups were matched in key physical attributes. However, consistent with diagnosis of atopic conditions, the AR group had significantly higher eosinophil counts than the control group (CG). The total WBC and lymphocyte counts were also signifi-

cantly elevated in the AR group, although the average counts were within normal reference ranges. The AR group had mild-moderate symptoms based on mRQLQ scores (2.84 ± 1.23 [out of a maximum possible score of 6]) with the majority sensitised to both plant pollens and dust mites. Several participants also reported allergies other than AR; a total of 44% of the cohort reported a history of skin allergies (eczema, hand dermatitis, urticaria, and itchy rash), 28% also reported a history of food allergy, and 12% also reported a history of drug allergy (including codeine [opioid], acetylsalicylic acid [non-steroidal anti-inflammatory drug], antibiotics, and metoclopramide [dopamine D_2 receptor antagonist/5-HT_3 receptor antagonist/5-HT_4 receptor agonist]).

Microbial Diversity

A trend for greater microbial diversity (~7%; p = 0.129) was noted among the CG. Higher operational taxonomic unit (OTU) count, CHAO1, and inverse Simpson α-diversity measures were observed in the CG compared to the AR group (Fig. 1). The Shannon diversity index, which considers both species richness and evenness, was significantly higher (p = 0.037) in the controls (CG) than in the AR group (Fig. 1).

Microbial Composition/Taxonomic Classification

Phyla

Taxonomic classification at the phylum level revealed that Firmicutes, Bacteroidetes, Verrucomicrobia, and Proteobacteria were the dominant bacterial phyla in both groups (Fig. 2). The Bacteroidetes (34.36% [27.67–43.21%] in AR vs. 28.74% [22.35–35.78%] in CG, U 425, p = 0.014) was significantly more abundant in the AR group than in the CG. In contrast, the Firmicutes phylum was significantly less abundant in the AR group than in the CG (57.24% [47.29–64.77%] vs. 64.44% [57.60–71.46%], U 396, p = 0.006). Further, there was a significant difference in the Firmicutes to Bacteroidetes ratio between the AR group and CG (1.73% [1.11–2.08%] vs. 2.33% [1.76–3.05%], U 377, p = 0.003).

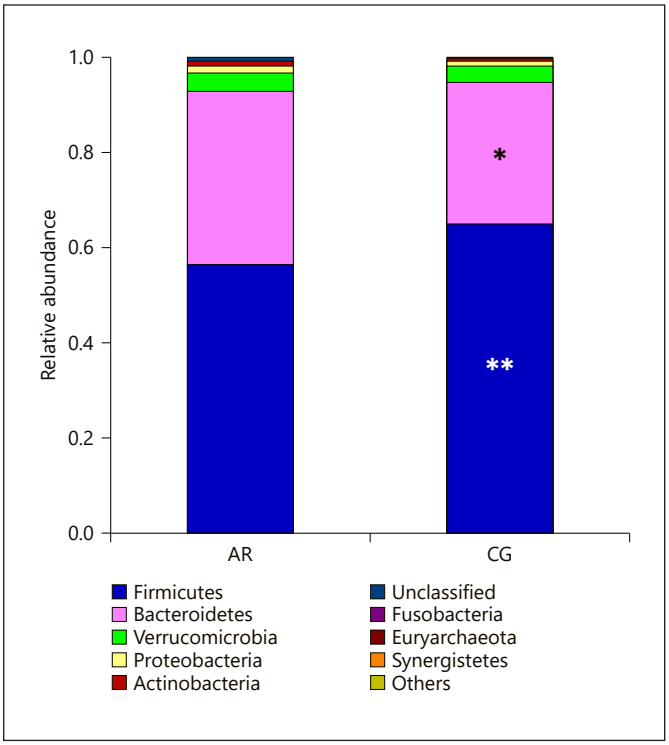


Fig. 2. The relative abundance of bacteria phyla for allergic rhinitis (AR) group and control group (CG) measured via 16s rRNA sequencing using collected stool samples. *p < 0.05, **p < 0.01.

Order

Within the order taxonomic rank, the OTUs obtained were assigned to 26 unique orders; of these, 12 orders were considered prevalent (detected in >50% of samples from either group) and 14 were less prevalent detected in <50% of samples), see online suppl. Table 1; see www.karger.com/doi/10.1159/000510536 for all online suppl. material. The most abundant order present in the both AR and CG cohorts were Clostridiales (53.14%), Bacteroidales (34.13%), Negativicutes unclassified (4.32%), and Verrucomicrobiales (3.76%). The order Bacteroidales was significantly more abundant in the AR cohort than in the CG cohort. In contrast, the Clostridiales was significantly less abundant in the AR cohort than in the CG cohort (Table 2).

Genus

At the genus level, 112 unique genera were identified; 57 of these genera were considered prevalent and 55 were less prevalent (online suppl. Table 2). The most abundant genera in both the AR and CG were *Bacteroides* (21.55%), *Faecalibacterium* (20.03%), Lachnospiraceae unclassified genera (6.25%), *Alistipes* (4.15%), *Akkermansia* (3.77%), *Prevotella* (3.71%), and *Oscillibacter* (3.51%). The genus *Parabacteroides* was significantly more abundant in the AR group than in the CG (Table 2). In contrast, *Coprococcus, Oxalobacter,* and *Sutterella* were significantly less abundant in the AR group (Table 2).

Among the less-prevalent genera, some differences in rates of detection between AR and CG were noted (Table 3). For example, the *Acidaminococcus* genus was de-

Table 2. Relative abundance (%) of differentially abundant taxa between the AR and CG at the order, genus and species level

	Mean and SD		Median and 25–75th percentile		*U* statistic	*p* value
	AR	CG	AR	CG		
Order						
Bacteroidales	36.30±12.10	28.70±7.97	34.36 (27.67–43.19)	27.68 (22.27–33.74)	385	0.004
Clostridiales	50.50±14.70	59.80±10.80	53.36 (40.59–59.33)	60.85 (55.22–67.28)	390	0.005
Genus						
Parabacteroides	2.40±2.55	1.15±1.03	1.20 (0.85–3.42)	0.75 (0.41–1.94)	406.5	0.008
Coprococcus	1.56±2.27	2.97±3.08	0.55 (0.17–2.07)	1.41 (0.52–5.45)	454	0.032
Oxalobacter	<0.01±<0.01	<0.01±0.01	<0.01 (<0.01–<0.01)	<0.01 (<0.01–0.01)	401	0.001
Sutterella	0.07±0.13	0.14±0.22	<0.01 (<0.01–0.10)	0.08 (0.01–0.18)	413	0.007
Faecalibacterium	19.30±12.10	21.80±11.50	18.51 (10–27.65)	25.14 (8.74–32.16)	574	0.386
Species						
Bacteroides massiliensis	0.66±1.19	1.24±1.72	<0.00 (0.00–1.06)	0.77 (<0.00–2.01)	464	0.029
Intestinimonas butyriciproducens	0.24±0.42	0.66±1.15	0.06 (0.02–0.23)	0.26 (0.05–0.67)	406.5	0.008
Eubacterium xylanophilum	0.11±0.14	0.27±0.30	0.05 (<0.00–0.14)	0.16 (0.08–0.44)	366	0.002
Murimonas intestini	0.01±0.01	0.02±0.03	<0.01 (<0.01–0.01)	0.01 (<0.01–0.03)	377	0.002
Clostridium asparagiforme	0.02±0.04	0.04±0.05	<0.01 (<0.01–0.01)	0.01 (<0.01–0.06)	461	0.025
Coprococcus eutactus	1.30±2.25	2.70±3.10	0.05 (<0.01–1.57)	1.27 (<0.01–5.32)	475.5	0.048
Oscillibacter valericigenes	0.23±0.33	0.45±0.47	0.12 (0.02–0.24)	0.25 (0.16–0.65)	408	0.008
Agathobaculum butyriciproducens	0.12±0.09	0.24±0.24	0.09 (0.05–0.16)	0.15 (0.08–0.30)	433	0.018
Anaerotruncus colihominis	0.03±0.03	0.02±0.01	0.02 (0.01–0.05)	0.01 (<0.01–0.03)	418	0.012
Oxalobacter formigenes	<0.01±0.01	0.01±0.01	<0.01 (<0.01–<0.01)	<0.01 (<0.01–0.01)	400	0.001
Sutterella wadsworthensis	0.05±0.11	0.09±0.19	<0.01 (<0.01–0.03)	0.02 (<0.01–0.09)	473	0.037

AR, allergic rhinitis; CG, control group.

tected significantly more frequently in the AR group than in the CG (~25% of AR samples vs. ~4% of non-AR samples). In contrast, the genera *Rothia* and *Coriobacteriaceae* unclassified were detected significantly more often in the CG than in the AR group.

Species

At the species level, 290 unique species were identified – 122 of these species were considered prevalent and 168 were less prevalent. *Anaerotruncus colihominis* was significantly more abundant in the AR group than in the CG (Table 2). In contrast, *Bacteroides massiliensis, Intestinimonas butyriciproducens, Eubacterium xylanophilum*, *Murimonas intestini*, *Clostridium asparagiforme, Coprococcus eutactus, Oscillibacter valericigenes*, *Agathobaculum butyriciproducens, Oxalobacter formigenes,* and *Sutterella wadsworthensis* were less abundant in the AR group than in the CG (Table 2).

Differences in the detection rate of less-prevalent species (<50% of either cohort) were observed between the AR and CG cohort (Table 3). *Clostridium hylemonae*, *Ruminococcus gnavus,* and *Acidaminococcus intestini* species were present significantly more in the AR cohort than in the CG. In contrast, *Rothia mucilaginosa*, *Muricomes intestini*, *Clostridium papyrosolvens*, *Clostridium straminisolvens,* and *Dialister succinatiphilus* were detected significantly less frequently in the AR cohort than in the CG.

PLS-DA Multivariate Analysis

Multivariate analysis using PLS-DA was performed on phylum to species level with microbiota abundance data. As shown in Figure 3a and b, PLS-DA discriminated the AR and CG cohorts based on the genus and species abundance data. Clustering of samples was observed at the genus and species levels for the first 2 principal coordinates, suggesting a distinct microbial structure between cohorts, although the observed clustering of samples accounts for a small amount of total variance.

Discussion

The current study analysed the faecal microbial community of adult AR sufferers and controls. The AR cohort had a distinct gut microbiome profile, marked by a re-

Table 3. Differentially detected taxa between the AR and CG at the family, genus, and species level

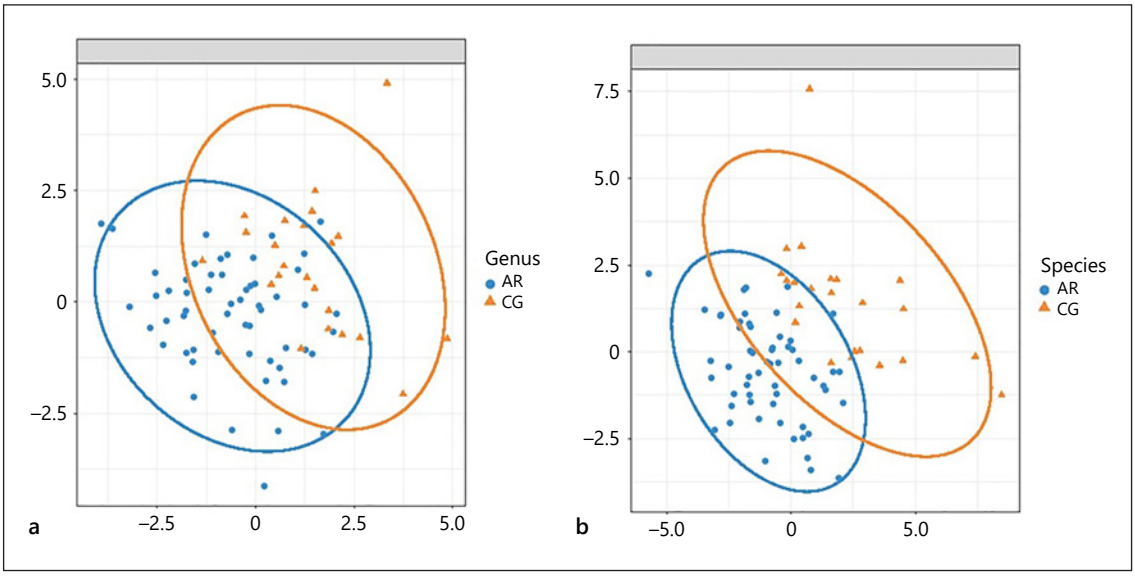
	AR *n* (%)	CG *n* (%)	Fisher's exact *p* value	χ^2 *p* value
Family				
Micrococcaceae	4 (7.02)	7 (30.43)	0.003	0.0001
Oxalobacteraceae	10 (17.54)	13 (56.52)	0.0009	0.0005
Genus				
Rothia	4 (7.02)	7 (30.43)	0.003	0.0001
Coriobacteriaceae unclassified	7 (12.28)	10 (43.48)	0.0049	0.0020
Acidaminococcus	14 (24.56)	1 (4.35)	0.0548	0.0360
Oxalobacter	10 (17.54)	13 (56.52)	0.0009	0.0005
Sutterella	25 (43.86)	19 (82.61)	0.0025	0.0016
Species				
Rothia mucilaginosa	4 (7.02)	7 (30.43)	0.0147	0.0092
Bacteroides massiliensis	23 (40.35)	16 (69.57)	0.0258	0.0180
Christensenella minuta	45 (78.95)	23 (100)	0.0154	0.0170
Muricomes intestini	0 (0)	2 (8.70)	0.0801	0.0242
Murimonas intestini	31 (54.39)	19 (82.61)	0.223	0.0183
Clostridium asparagiforme	23 (40.35)	15 (65.22)	0.0517	0.0438
Clostridium hylemonae	27 (47.37)	4 (17.39)	0.0212	0.0127
Ruminococcus gnavus	26 (45.61)	5 (21.74)	0.0748	0.0473
Clostridium papyrosolvens	5 (8.77)	6 (26.09)	0.0687	0.0418
Clostridium straminisolvens	4 (7.02)	8 (34.78)	0.0036	0.0016
Acidaminococcus intestini	13 (22.81)	1 (4.35)	0.0568	0.0492
Dialister succinatiphilus	9 (15.79)	9 (39.13)	0.0371	0.0237
Oxalobacter formigenes	10 (17.54)	13 (56.52)	0.0009	0.0005
Sutterella wadsworthensis	23 (40.35)	16 (69.57)	0.0258	0.0180

In a given sample, taxa were considered "detected" if the relative abundance was >0% and "not detected" if the relative abundance was 0%. AR, allergic rhinitis; CG, control group.

duced microbial diversity and altered abundance of certain gut microbes compared to the controls. The results presented here provide evidence that unique gut microbial patterns occur in adult AR sufferers and warrant further investigation in the form of mechanistic studies.

A key finding from the current study was related to overall microbial diversity. Species richness (α-diversity) measured via the Shannon index was significantly reduced in the AR cohort compared to the CG. A similar trend of reduced species richness in the AR cohort was also observed with the inverse Simpson, observed OTU counts, and CHAO1 diversity indices. Lack of sufficient richness or evenness in the gastrointestinal microbial community appears to impair its ability to withstand exogenous disturbances [28]. Indeed, it has been suggested that reliable microbial richness indices may be useful indicators to determine the relative stability or "fitness" of the gut microbiome [28]. Other studies have also identified reduced richness in atopic individuals compared to controls. Hua et al. [10] examined publicly available 16S rRNA data collected from the American Gut Project and reported that species richness was significantly negatively associated with self-reported seasonal allergy in adult sufferers. Similarly, Bisgaard et al. [29] reported that reduced faecal bacterial diversity at 1 and 12 months after birth significantly increased the risk of developing allergic sensitisation and AR by the age of 6 years ($n = 346$).

Other key findings from the current study relate to the differential abundance/detection of particular microbial taxa or specific microbes in the AR group compared to the controls. At the phylum level, a significantly different Firmicutes to Bacteroidetes ratio between groups, with a higher abundance of Bacteroidetes and lower abundance of Firmicutes in the AR cohort were detected. Increased abundance of Bacteroidetes in the AR cohort translated to the class (Bacteroidia) and order (Bacteroidales) taxa. Other studies of atopic cohorts have also identified a higher abundance of Bacteroidetes classifications in stool samples from atopic subjects. Analysis of data from the American Gut Project identi-



Color version available online

Fig. 3. Multivariate analysis using partial least squares-discriminant analysis at the genus and species level collated from 16s rRNA sequencing data performed using collected stool samples. **a** At the genus level, clustering of samples within the AR and CG cohorts was observed for the first 2 principal coordinates. The first coordinate represents 7% variance explained and second coordinate represents 6% variance explained. **b** At the species level, clear clustering of allergic rhinitis (AR) and control group (CG) samples was also observed for the first 2 principal coordinates. The first coordinate represents 7% of variance explained and the second coordinate represents 4% of variance explained. The blue full circles represent the AR samples and the orange full triangles represent the CG samples. The coloured ellipses, blue for AR and orange for CG, are plotted to represent the 95% confidence level of the population.

fied a higher abundance of Bacteroidales in adults with self-reported nut and seasonal pollen allergies [10]. In a small study of Japanese infants (n = 15), the abundance of Bacteroidaceae was significantly higher in infants who later went on to develop atopic disease by the age of 2, as determined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [30]. It is worth noting that Bacteroidetes and Firmicutes are the most abundant phyla present in the western faecal microbiome [31, 32], and this observation was also reflected in this study. The metabolites generated by these phyla play a significant role in colonic health and immune regulation. The Bacteroidetes phyla are generally associated with a greater production of acetate and propionate, whereas the Firmicutes phyla are associated with a greater production of butyrate [33]. Butyrate is a key energy source for colonic epithelial cells and contributes to maintaining the intestinal barrier via modulation of tight junction expression [34]. A dysfunctional gut barrier allows for increased pro-inflammatory molecules and antigen transfer into submucosa and systemic circulation, resulting in local and systemic inflammatory responses. A dominance of Bacteroidetes over Firmicutes may reduce overall butyrate production and thereby affect the integrity of the gut barrier. Interestingly, clinical and experimental studies have shown that gut permeability is increased in subjects with allergic disease compared to healthy controls [35–40]. Given that these results were generated from independent studies, future work is needed to explore the potential link between abundance of butyrate-producing bacteria and gut permeability in atopy.

At the class and order level, this study reports a reduced abundance of Clostridia/Clostridiales (Firmicutes) in the AR cohort compared to the controls. A reduced abundance of Clostridiales was also observed by Hua et al. [10] in adults with self-reported nut and seasonal pollen allergies. Reduced Clostridia has also been detected in faecal samples of atopic infants and children. Candela et al. [41] reported a lower count of *Clostridium IV* in their cohort of Italian children (n = 19 allergic, 12 non-allergic; aged 4–14 years). Verhulst et al. [42] observed an association between reduced prevalence of *Clostridium* in stool samples of infants (n = 154) at 3 weeks of age and the occurrence of wheezing symptoms at 12 months of age. Indigenous intestinal *Clostridium* clusters IV, XIVa, and

XVIII have been recognised as effective inducers of Tregs in the colon of mice [43, 44]. Tregs are known for their ability to maintain immune homeostasis and promote immune tolerance to allergens, which is particularly relevant in the pathogenesis of allergic diseases [45]. Notably, reduced Clostridia has also been identified in other immune-mediated diseases such as Crohn's disease [46].

At the genus level, we observed a higher abundance of *Parabacteroides* (Bacteroidetes) and a reduced abundance of *Coprococcus* (Firmicutes), *Sutterella* (Proteobacteria), and *Oxalobacter* (Proteobacteria) in the AR group compared to the CG. No direct associations between the faecal abundance of the butyrate-producing *Coprococcus* species and adults with AR have been previously reported. However, Simonyté Sjödin et al. [47] reported an underrepresentation of *Coprococcus* abundance in stool samples from children with a history of allergic disease (21 allergic, 72 non-allergic) from infancy to 8 years of age. While we emphasise the link between this bacterial genus and AR, a reduced abundance of *Coprococcus* has also been reported in adults with other inflammatory conditions such as ESRD [48] and psoriatic arthritis [49]. The current study is the first to report a link between AR and *Sutterella* abundance in adult stool samples. The *Sutterella* species have been reported to be highly prevalent in the gut mucosa of humans possibly due to their ability to resist bile acids [50]. A reduced *Sutterella* abundance has also been reported in other atopic conditions such as IgE-associated eczema in infants ($n = 20$) compared with non-atopic infants ($n = 20$) at 1 and 12 months of age. A further interesting and novel finding in the present study was the reduced abundance of the genus *Oxalobacter* (Proteobacteria) in the AR cohort. Members of the Oxalobacteraceae are known to colonise the rhizosphere and roots of many plant species [51]. In relation to human health, *Oxalobacter* species metabolise oxalate in the intestinal tract and is protective against the formation of calcium oxalate kidney stones and other oxalate-associated pathologies [52]. Furthermore, a link between the presence of Oxalobacteraceae in house dust and the prevalence of atopy has been observed. Indeed, members of the Oxalobacteraceae were found to be more abundant in dust samples from the Finnish Karelia homes compared to geographically adjacent Russian Karelia, whereby the abundance of atopic disease in this region is 4-fold lower [53]. While microbial gut composition was not performed in allergy sufferers living in these regions, these findings provide a potential link between exposure to plant-related microbes such as Oxalobacteraceae and the prevalence of atopy.

In the current study, numerous species were differentially abundant/detected (11 abundant/14 detected) in the AR group compared to the controls. Among these species, several were identified as previously associated with atopy; however, for a large proportion of the differentially abundant/detected species, the relationship with atopy is unknown and warrants further investigation. Species differentially abundant/detected and of particular interest include *S. wadsworthensis* (Proteobacteria), *C. eutactus* (Firmicutes), and *R. gnavus* (Firmicutes). The finding of a reduced abundance of *S. wadsworthensis* and *C. eutactus* in the AR group is consistent with a previous report in an atopic cohort. In a study of adults residing in the UK with asthma ($n = 36$ asthma, 185 controls), a reduced enrichment of *S. wadsworthensis* and *C. eutactus* was observed in the asthma group compared with the controls [54]. Interestingly, *C. eutactus* produces the short chain fatty acid butyrate, which is a known inducer of colonic regulatory T cells [55]. The authors suggest that the depletion of butyrate-producing bacteria, such as *C. eutactus*, may be linked with the presentation of asthma. In the current study, a significantly increased detection of *R. gnavus* in the AR group when compared to the CG was observed. *R. gnavus* has been previously associated with the development and pathogenesis of atopy, especially respiratory allergies [56]. Chua et al. [56] reported a higher incidence of *R. gnavus* in stool specimens from infants who later developed respiratory allergies. In a follow-up experimental asthmatic mouse model, ovalbumin-sensitised/challenged mice were infected with *R. gnavus* via oral gavage intragastric administration. The *R. gnavus*-infected mice showed greater secretion of interleukin (IL)-25, IL-33, and thymic stromal lymphopoietin by colonic tissues, thereby promoting Th2 differentiation and further cytokine release, and an enhanced infiltration of eosinophils and mast cells to the colon and lung parenchyma [56]. In addition, the *R. gnavus*-infected mice displayed increased airway hyperresponsiveness and histologic airway inflammation [56], providing evidence of a clear link between gut bacterial species and mechanisms underpinning allergic disease.

Key differentially abundant species identified in previous reports of atopic children and infants, including *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bifidobacterium catenulatum*, *Bifidobacterium longum*, *Staphylococcus aureus*, *Bacteroides fragilis*, *Clostridium difficile, Bacteroides Vulgatus,* and *Escherichia coli*, were not significantly differentially abundant in this cohort of adults suffering AR. This finding may be due to differences in study features, cohort ethnicity,

sample processing methodology, and microbial identification tools used. Nonetheless, the differentially abundant microbes present in infancy/childhood cannot be entirely extrapolated to adults and therefore demonstrates the importance of sampling the microbiome of allergy sufferers in adulthood. Longitudinal studies that capture the early microbiome and the microbiome throughout childhood to adulthood, while a challenging task, are worth further investigation to elucidate shifts in the microbiome of allergic subjects over time. In addition, mechanistic studies in an animal model such as gnotobiotic mice could be conducted to elucidate how the taxa identified in the current study contribute to the pathophysiology of AR.

The strengths of the current study lie in the novelty of assessing gut microbiome composition in an adult population with clinically well-characterised allergic disease. In addition, notwithstanding population studies, the sample size in this investigation is larger than typical single-centre investigations. Despite the strength of the design, the authors acknowledge that this study is not without its limitations. These particularly relate to the use and interpretation of 16s rRNA amplicon analysis in providing high resolution of the microbial population at the species level. Nevertheless, 16s rRNA sequencing is often employed in human microbiome studies due to its ability to resolve the microbial population structure and biodiversity and its relative affordability. Additionally, this study describes the microbial composition of stool samples from adults with AR and adults without AR. Analysis of stool samples has the limitation of capturing luminal microbiota and not the mucosal-associated microbiota, which may play a critical role in regulation of the mucosal immune system and local mucosal immune regulation relevant in AR. However, without invasive procedures, there are no real alternatives. Cohort factors such as lifestyle, diet, and age were also not evaluated in this report, and therefore, the effect of these variables on the gut microbiome could not be quantified as this study was not sufficiently powered to conduct these analyses. However, a post hoc analysis to examine the impact of co-existing skin allergies on the gut microbiome was undertaken. Approximately 11% (6/57) of the AR group reported a personal history of urticaria and 12% (7/57) reported a personal history of eczema. Removing participants in the AR group with either a personal history of urticaria or eczema did not change the observations at the phyla, order, and genus level. In addition, post hoc correlation analyses of the AR group were performed with Spearman's test to examine the effect of mRQLQ scores on the gut microbiome. No significant correlation was observed between the mRQLQ scores and diversity indices or relative abundance of taxa at the phyla, order, genus, and species. To prevent the confounding effect of antibiotic exposure on the gut microbiome, participants who had taken antibiotics in the 30 days prior to enrolment were excluded from participation. However, it should be noted that the effect of antibiotic usage prior to this period (>30 days prior to enrolment) was not evaluated in this study. The relationship between the immune parameters and the gut microbiome composition was not explored in this study. Mechanistic studies are needed to better understand the effect of specific gut microbial taxa and microbiota population structures on key effector cells and mediators involved in the allergic response.

Overall, a unique microbial community in the AR cohort, marked by a reduced microbial diversity, increased abundance of Bacteroidetes and *Parabacteroides*, and a reduced abundance of *Oxalobacter, Sutterella, Coprococcus,* and Clostridiales was observed in the current study. Several taxa identified in our study were consistent with previous reports in atopic adults. However, this study also identified taxa that were unique to our study and have not been previously associated with atopy. Interestingly, the differentially abundant/detected taxa reported here were not always consistent with the findings presented in atopic paediatric cohorts. In light of the unique microbiome patterns in adult AR subjects presented here, identifying the metabolites and mechanisms underpinning the microbiota-host relationship will improve the understanding of how the composition of the microbiome regulates immune homeostasis and may advise potential therapeutic options for treating allergies (e.g., dietary intervention, probiotics, and faecal transplant).

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Statement of Ethics

This study was approved by the Griffith University Human Research Ethics Committee (Approval Nos. 2015/564/HREC; 2016/279). All subjects provided written informed consent prior to participation.

Conflict of Interest Statement

The authors report no conflicts of interest in relation to this work.

Author Contributions

A.M.W., N.P.W., and A.J.C. designed the study. A.M.W. performed the experiments and data analysis. P.Z. performed the PLS-DA statistical analysis. A.M.W. and A.J.C. drafted the manuscript. A.W.C., N.P.W., P.Z., and P.K.S. revised the manuscript. All authors approved the final version of the paper.

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