Volume 10:1-14

UJEMI+

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Eczema May Be Masking the Effects of Multiple Sclerosis on the Gut Microbiome

Clare Konnert, Kaitlin Law, Michelle Tong, Anny Xia, Davey Li

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Multiple sclerosis is a chronic autoimmune-mediated neurodegenerative disease with no cure leading to physical disability and cognitive impairment. Eczema patients often have an altered gut microbiota and increased susceptibility to autoimmune illnesses such as multiple sclerosis. In addition, these patients with multiple sclerosis and eczema are generally burdened with taking a higher number of medications, which can impact gut microbial diversity. However, the impact of eczema on multiple sclerosis and corresponding medication intake in treatment has yet to be fully established. Here, we investigate gut microbiome composition in multiple sclerosis patients and healthy controls with and without eczema that take varying medication types. QIIME2 was utilised to process 16S rRNA gut microbiome sequences from the International Multiple Sclerosis Microbiome Study prior to analyses on alpha and beta diversity, core microbiome, and indicator species with R. Interestingly, significant differences in beta diversity between multiple sclerosis patients and healthy controls were only observed if patients did not have eczema. Similarly, significant beta diversity differences between patients taking or not taking over-the-counter medication were only observed if patients did not have eczema. Eczema was concluded to potentially mask the effects of multiple sclerosis and over-the-counter medication use, creating a mediating effect on the gut microbiome. Indicator taxa analysis of multiple sclerosis patients reveals 28 indicator species for eczema, suggesting a unique gut microbial community in eczema patients. This suggests that additional consideration should be taken when deciding treatment courses for multiple sclerosis patients with and without eczema.

INTRODUCTION

Multiple sclerosis (MS) is a chronic neurodegenerative disease of the central nervous system where autoimmunity and focal inflammation result in demyelination and axonal transection (1). Currently, there is no cure for MS due to the disease's complexity and dynamics (1). Existing treatments only reduce disease activity and progression but do not provide a cure. (2). Mechanisms underlying the development and pathogenesis mechanisms of MS have yet to be fully elucidated prompting interest in understanding lifestyle and environmental factors that shape this disease (3). Several investigations have demonstrated that the gut microbiome can impact neuroinflammatory disease, driving interest in understanding how specific microbiota compositions may affect the development and course of MS (4–12).

Previous work on the association between the gut microbiome and MS performed by the International Multiple Sclerosis Microbiome Study (iMSMS) revealed MS patients had an increase in bacteria exerting pro-inflammatory effects on T cells, elevation of specific taxa that facilitate molecular mimicry, and depletion of potentially beneficial bacteria resulting in perturbation of key metabolic pathways (13). A closer examination of the analysis performed

Published Online: September 2024

Citation: Konnert, Law, Tong, Xia, Li. 2024. Eczema may be masking the effects of multiple sclerosis on the gut microbiome. UJEMI+ 10:1-14

Editor: Ronja Kothe, University of British Columbia

Address correspondence to:
https://jemi.microbiology.ubc.ca/

on the iMSMS microbiota dataset and existing work in the literature revealed limited investigations on the impact of atopic dermatitis (eczema) and healthy controls on gut microbial composition in MS patients who take different classes of medications (prescription vs. over-the-counter; OTC).

Eczema and various medications have been observed to cause diverse and varying impacts on microbiota composition, with gut dysbiosis serving as a central factor in generating pro-inflammatory T cells and auto-antigen specific lymphocytes (7, 14, 15). Eczema may increase susceptibility to autoimmune disease and mediation consumption associated with eczema can result in loss of homeostasis, perpetuating inflammation (16–22). Treatment of eczema in patients with MS is difficult, particularly in the case of ongoing immunosuppressive treatment that contraindicates the treatment of eczema (23–27). MS and eczema patients are often burdened with polypharmacy, involving the use of multiple medications, which contributes to altered gut microbial diversity (26–32). Furthermore, aggressive prescription medications can more drastically perturb the gut microbiome resulting in dysbiosis, microbial translocation and epithelial damage (21–23, 33). Previous *in vitro* characterization has also shown that acetaminophen (Tylenol®), the most common OTC medication, did not negatively impact the bacterial growth of common gut species (34). Thus, we aimed to examine the impact of eczema on MS patients, both those taking or not taking OTC medication, as we expect eczema to differentially impact patients' gut microbiome, which in turn could influence MS outcomes (22, 35).

With the microbiome mediating many factors that influence the outcomes of MS patients, we aimed to investigate how eczema influences the gut microbiota compositions of healthy individuals and those with MS who take different classes of medications. Our work has the potential to contribute to a better understanding of the interplay between skin conditions, microbiome health, and potential MS therapeutic interventions. This research characterizes specific microbial communities in MS patients, and highlights additional factors to consider when devising treatment plans for MS patients both with and without eczema.

METHODS AND MATERIALS

Dataset overview. The dataset utilised in this study was generated by the International Multiple Sclerosis Microbiome Study (iMSMS) Consortium (13). Patients were recruited through MS clinics at UCSF (San Francisco, CA), Brigham and Women's Hospital (Boston, MA), Mount Sinai (New York, NY), the Anne Rowling Clinic (Edinburgh, UK), University of Pittsburgh (Pittsburgh, PA), Biodonostia Health Research Institute (San Sebastián, Spain) and FLENI (Buenos Aires, Argentina). A total of 576 MS patients and their corresponding healthy household control (HHCs) recruited into two cohorts. Cohort 1 had 128 sets of MS and control pairs, and Cohort 2 had 448 sets. Participants with diagnosed MS and an unrelated household control that they had cohabited with for a minimum of 6 months were eligible for inclusion. Participants were also required to be of White ethnicity (Hispanic or non-Hispanic) to match the characteristic genetic risk profile of MS (13). Individuals were excluded if they had been prescribed oral antibiotics in the preceding 3 months, corticosteroids in the past 30 days, were on disease modifying therapies (DMT) for less than 3 months or had any other autoimmune disorders, neurological disorders, or gastrointestinal infections.

Study Cohort. Only individuals in Cohort 2 who had never smoked were included in this study to control for confounding variables. A new metadata category "ms_and_eczema" was created in preparation for downstream analyses. All metadata manipulation and analyses performed with R in RStudio (version 4.2.2) can be found in the supplemental R script (RScript).

Dataset processing using the QIIME2 pipeline. The modified metadata was imported into QIIME2 along with the modified manifest file for data processing (36). All sequences were demultiplexed and denoised using the Diverse Amplicon Denoising Algorithm 2 (DADA2) open-source software package developed by Callahan et al. (13, 37). Trimming was not necessary as the quality score of all nucleotides was over 30. The Silva 138-99 classifier dataset (38–40) was trained using V4 primer sequences (forward: GTGCCAGCMGCCGCGGTAA, reverse: GGACTACHVGGGTWTCTAAT) with a

truncation length of 151 (41). The denoised ASVs were then aligned to taxonomic information using the trained classifier and filtered to remove nonbacterial mitochondria and chloroplast sequences. The rarefaction depth was determined using the MS & eczema category in the modified metadata. The sampling depth was set to 8788 to retain all 25 of the control and eczema samples as well as the 19 MS and eczema samples. Additional details regarding data processing performed can be found in the supplemental QIIME2 script (ShellScript).

Data processing in R/RStudio. Processed data were exported for further analysis and converted to Phyloseq objects in R using the Phyloseq package (42). Samples were then filtered for individuals between the ages of 20 and 50 as this is the most common age range for MS onset (43).

Alpha and beta diversity analyses. Alpha and beta diversity analyses were conducted on R/RStudio using the Tidyverse, Vegan, Ape, and Phyloseq packages (42, 44–46). Shannon and observed features diversity metrics were visualised as boxplots using ggplot2 as a metric of alpha diversity (47). Statistical analyses were performed using the Wilcoxon rank-sum test with p-values equal or less than 0.05 representing statistical significance. Beta diversity analyses were conducted using Bray and Jaccard diversity metrics and visualised as principal coordinates analysis (PCoA) plots, showing the percentage of total variance on each axis. Statistical analyses were conducted using the permutational multivariate analysis of variance (PERMANOVA) test with p-values equal or less than 0.05 representing statistical significance. All diversity analyses performed can be found in the supplemental R script (RScript).

Core microbiome analysis. Core microbiome analysis was performed using the Tidyverse, Phyloseq, Microbiome, Ggvenn, RColorBrewer, Reshape2, Ggplot2, and Knitr packages in R/RStudio (42, 44, 47–52). The Phyloseq object was subsetted for MS patients with and without eczema to compare microbiome differences between OTC medication usage in each group. To balance selectivity and maintain a sufficient core taxa pool, a detection threshold of 0.001 and 0.50 minimum prevalence parameters was applied. Core microbiome taxa were identified for the following subsets—MS patients without eczema taking and not taking OTC, and MS patients with eczema taking and not taking OTC. Shared and distinct core taxa for these subsets were visualised using Venn diagrams.

Indicator species analysis. Indicator species analysis was performed using the Indicspecies, Tidyverse and Phyloseq packages in R/RStudio. Taxonomic groups significantly associated with MS patients with eczema, and MS patients taking OTC medication were identified (42, 44, 53). The Phyloseq object was transformed into compositional relative abundance and grouped at the genus level to be used as predictors within the multipatt function for analysis. Species were filtered for significance with a p-value equal to or less than 0.05.

RESULTS

The effect of MS on microbial diversity is only apparent in patients without eczema. To investigate differences in microbial diversity associated with MS (healthy control and MS) and eczema status, we conducted alpha (observed taxa and Shannon diversity) and beta (Bray-Curtis and Jaccard) diversity analyses. Beta diversity analyses using Bray-Curtis and Jaccard metrics reveal distinct clustering based on eczema status when visualised by PCoA plot (Figure 1). MS status affects microbial diversity only in non-eczema patients when characterised using the Bray-Curtis and Jaccard dissimilarity index (Figure 1). This suggests that microbial diversity differences attributed to disease status are only visible in individuals without eczema. Notably, no significant alpha diversity differences are linked to disease or eczema status (Figure S1). The observed features and Shannon diversity metrics in alpha diversity evaluates richness, and richness plus abundance respectively. This is analogous to the Jaccard index and Bray-Curtis metrics for beta diversity, which also considers community composition differences across groups. Hence, the microbial diversity differences in Jaccard and Bray-Curtis metrics indicate that MS induces alterations in the richness and abundance

of taxa, resulting in a distinct diversity profile that significantly differs from that of healthy individuals. However, the impact of microbiome changes due to eczema confounds this effect.

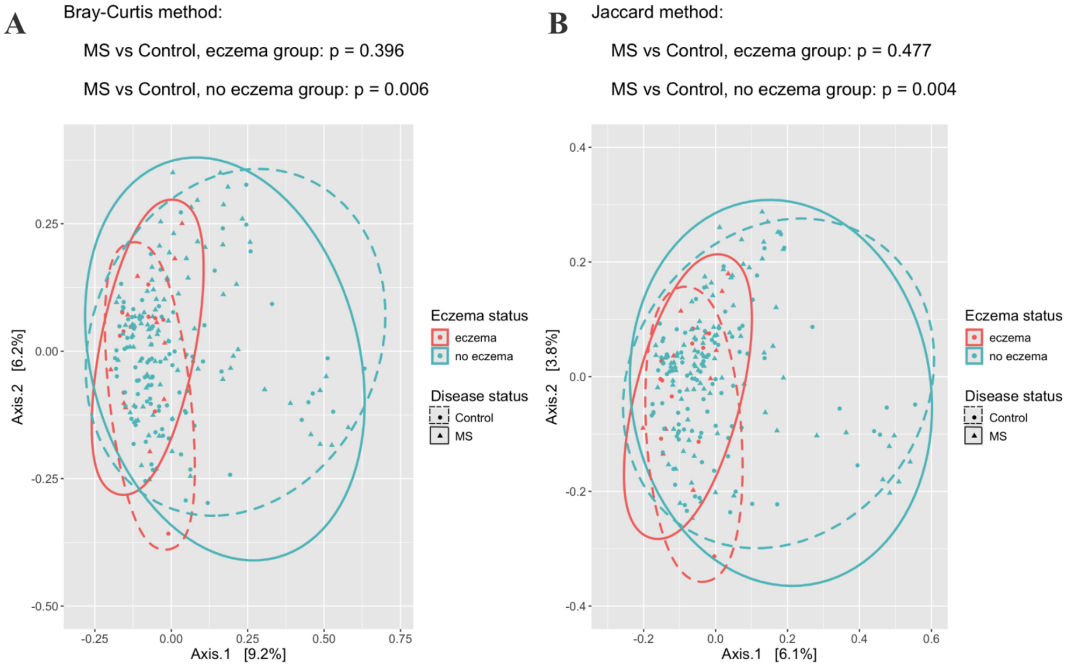

FIG. 1 MS affected gut microbial diversity in patients without eczema. PCoA plot visualising beta microbial diversity using the (A) Bray-Curtis and (B) Jaccard metrics with corresponding p-values. Statistical analysis was performed using the PERMANOVA test.

Medication class has no significant impact on microbial diversity in MS patients. To investigate if the medication class (prescription and OTC medication) impacts the microbial diversity of MS patients, alpha (observed taxa and Shannon diversity) and beta (Jaccard and Bray-Curtis) diversity analyses were performed. When MS patients are subsetted for medication class, Bray-Curtis and Jaccard diversity metrics revealed no distinct clustering when visualised using a PCoA plot which indicates no microbial diversity differences (Figure 2). The same trend is observed in alpha diversity analyses (Figure S2). Hence, medication usage is concluded to have no impact on microbial diversity in MS patients.

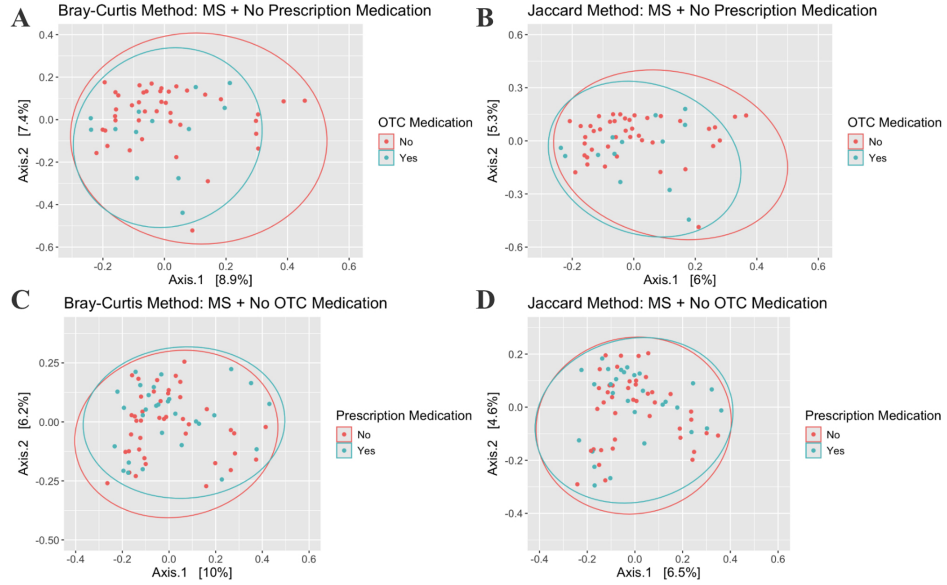

FIG. 2 Medication class does not impact microbial diversity. PCoA plot visualising microbial diversity for MS patients not taking (A,B) prescription medication and (C,D) OTC medication. (A,C) Bray-Curtis and (B,D) Jaccard diversity analyses reveal no statistically significant differences. Statistical analysis was performed using the PERMANOVA test.

Effect of OTC Medication on microbial diversity is only visible in MS patients without eczema. As our diversity analyses indicated minimal impact of medication class on microbial diversity in MS patients, we investigated whether eczema impacts the effect of medication class in MS patients. Beta diversity analysis using Bray-Curtis and Jaccard metrics revealed that eczema status does not impact microbial diversity concerning prescription medication usage in MS patients (Figure S3). Distinct clustering based on eczema status was observed for MS patients not taking prescription medication when visualised using a PCoA plot (Figure 3). Hence, revealing a significant difference in microbial diversity associated with OTC usage status for MS patients without eczema only (Figure 3). This suggests that OTC medication usage has a greater impact on the microbial diversity, abundance and richness of MS patients without eczema than those with eczema. No alpha diversity differences in observed taxa or Shannon diversity attributed to OTC medication status were observed in MS patients with or without eczema (Figure S4).

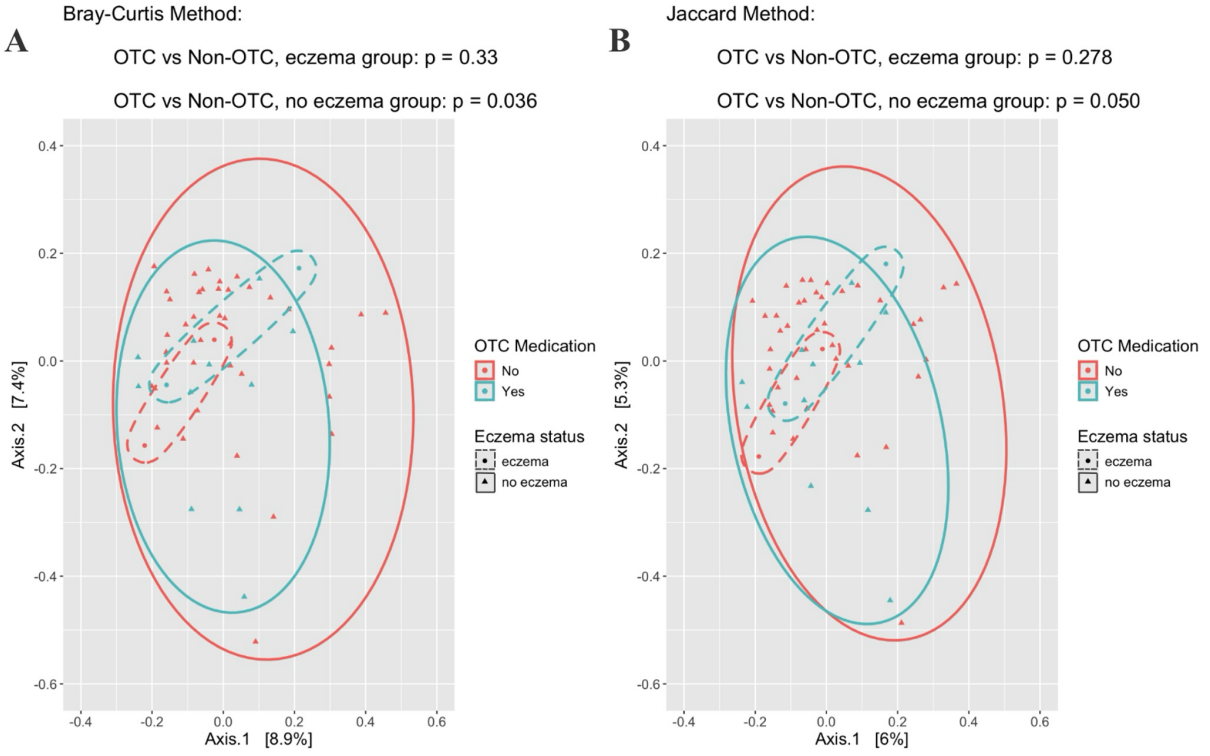

FIG. 3 The impact of over-the-counter (OTC) medication on microbial diversity is only evident in MS patients without eczema. Included MS patients are not taking prescription medication. (A) Bray-Curtis and (B) Jaccard beta diversity metrics were used. Statistical analysis was performed using the PERMANOVA test.

Unique taxonomic profiles are associated with eczema or taking OTC medication. An indicator species analysis was performed to determine the number of unique indicator taxa in MS patients with and without eczema who were or were not taking OTC medications. In order to remove the potential confounding effect of prescription medication, the analysed patients were not taking prescription medication. 28 and 21 unique genera were associated with having eczema and taking OTC medication, respectively (Table 1). There is one indicator taxa that is associated with both having eczema and taking OTC medication (Table 1).

TABLE. 1 Distinct indicator taxa of MS patients with eczema or using OTC medication. Indicator species analysis was conducted on fecal samples of MS patients and healthy controls (n = 481). Taxonomic level was filtered at the genus level, and only significant uniqueness is illustrated with increasing observed indicator value ($p<0.05$). Observed indicator value represents the overall probability that the particular ASV will be observed in both eczema and non-eczema patients. Results indicate 28 genera unique to eczema and 21 genera unique to OTC medication usage, with no indicator taxa observed for patients without eczema or not taking OTC medication.

Phylum	Genus	Species	Eczema status	OTC Medication Status	Observed Indicator Value	P-value
Bacteroidota	Bateroides	bacteroides_fragilis	eczema	N/A	0.79	0.05
Bacteroidota	Parabacteroides	parabacteroides_distasonis	eczema	N/A	0.54	0.045
Bacteroidota	Prevotella	uncultured_organism	eczema	N/A	0.42	0.035
Bacteroidota	Parabacteroides	parabacteroides_goldsteinii	eczema	N/A	0.40	0.045
Firmicutes	Lachnoclostridium	uncultured_organism	eczema	N/A	0.77	0.010
Firmicutes	Lachnospiraceae	lachnoclostridium	eczema	N/A	0.77	0.010
Firmicutes	Oscillospiraceae	flavonifractor	eczema	N/A	0.70	0.025
Firmicutes	Lachnospiraceae	lachnospiraceae_UCG-004	eczema	N/A	0.68	0.040
Firmicutes	uncultured	clostridium_sp.	eczema	N/A	0.64	0.005
Firmicutes	Lachnoclostridium	human_gut	eczema	N/A	0.64	0.010
Firmicutes	Lachnospiraceae_UCG-008	uncultured_organism	eczema	N/A	0.61	0.010
Firmicutes	Lachnospiraceae	lachnospira	eczema	N/A	0.60	0.030
Firmicutes	Erysipelotrichaceae	holdemania	eczema	N/A	0.55	0.020
Firmicutes	Anaerosporobacter	uncultured_organism	eczema	N/A	0.54	0.020
Firmicutes	Lachnospiraceae_FCS020_group	uncultured_Clostridium	eczema	N/A	0.52	0.005
Firmicutes	Veillonellaceae	veillonella	eczema	N/A	0.49	0.030
Firmicutes	Erysipelatoclostridium	erysipelatoclostridium_ramosum	eczema	N/A	0.46	0.025
Firmicutes	uncultured	uncultured_bacterium	eczema	N/A	0.45	0.10
Firmicutes	Clostridia_vadinBB60_group	uncultured_bacterium	eczema	N/A	0.42	0.030
Firmicutes	Clostridia_UCG-014	clostridia_UCG-014	eczema	N/A	0.42	0.015
Firmicutes	Lactobacillaceae	lactobacillus	eczema	N/A	0.42	0.020
Firmicutes	Roseburia	gut_metagenome	eczema	N/A	0.42	0.030
Firmicutes	Butyricicoccus	uncultured_bacterium	eczema	N/A	0.41	0.015
Firmicutes	Phocea	ucultured_bacterium	eczema	N/A	0.41	0.015
Firmicutes	Butyricicoccus	uncultured_bacterium	eczema	N/A	0.41	0.015
Firmicutes	Fusicatenibacter	uncultured_bacterium	eczema	N/A	0.41	0.030
Proteobacteria	Oxalobacter	oxalobacter_formigenes	eczema	N/A	0.58	0.005
Actinobacteriota	Corynebacteriaceae	corynebacterium	N/A	Yes	0.57	0.010
Actinobacteriota	Brevibacterium	brevibacterium_ravenspurgense	N/A	Yes	0.48	0.025
Bacteroidota	Parabacteroides	parabacteroides_distasonis	N/A	Yes	0.56	0.035
Fimicutes	[Eubacterium]_xylanophilum_group	uncultured_organism	N/A	Yes	0.71	0.040
Fimicutes	Lachnospiraceae	blautia	N/A	Yes	0.64	0.005
Fimicutes	Ruminococcaceae	subdoligranulum	N/A	Yes	0.61	0.010
Fimicutes	Ruminococcaceae	faecalibacterium	N/A	Yes	0.60	0.010
Fimicutes	Lachnospiraceae	lachnospiraceae_UCG-001	N/A	Yes	0.58	0.035
Fimicutes	Lactobacillus	lactobacillus_iners	N/A	Yes	0.55	0.015

Fimicutes	Lachnospirales	lachnospiraceae	N/A	Yes	0.52	0.020
Fimicutes	Clostridia_vadinBB60_group	metagenome	N/A	Yes	0.47	0.010
Fimicutes	UCG-010	UCG-010	N/A	Yes	0.46	0.025
Fimicutes	Turicibacter	turicibacter	N/A	Yes	0.46	0.045
Fimicutes	Peptostreptococcales-Tissierellales	anaerococcus	N/A	Yes	0.45	0.030
Fimicutes	Peptostreptococcales-Tissierellales	gallicola	N/A	Yes	0.45	0.035
Fimicutes	Clostridia_vadinBB60_group	uncultured_bacterium	N/A	Yes	0.39	0.030
Fimicutes	UCG-010	metagenome	N/A	Yes	0.39	0.030
Proteobacteria	Enterobacterales	enterobacteriaceae	N/A	Yes	0.54	0.010

More shared taxa are noted in MS patients with eczema who were and were not using OTC medications compared to those without eczema. A core microbiome analysis was performed to determine the number of shared taxa in MS patients with and without eczema who were or were not taking OTC medications. Between MS patients without eczema taking and not taking OTC medication, 39 shared taxa were identified, corresponding to a 91% overlap (Figure 4). Between MS patients with eczema taking and not taking OTC medication, 24 shared taxa were identified, corresponding to a 40% overlap (Figure 4). Taken together, the results indicate that there are more shared taxa among MS patients who did not have eczema regardless of whether they were taking OTC medication (Figure 4).

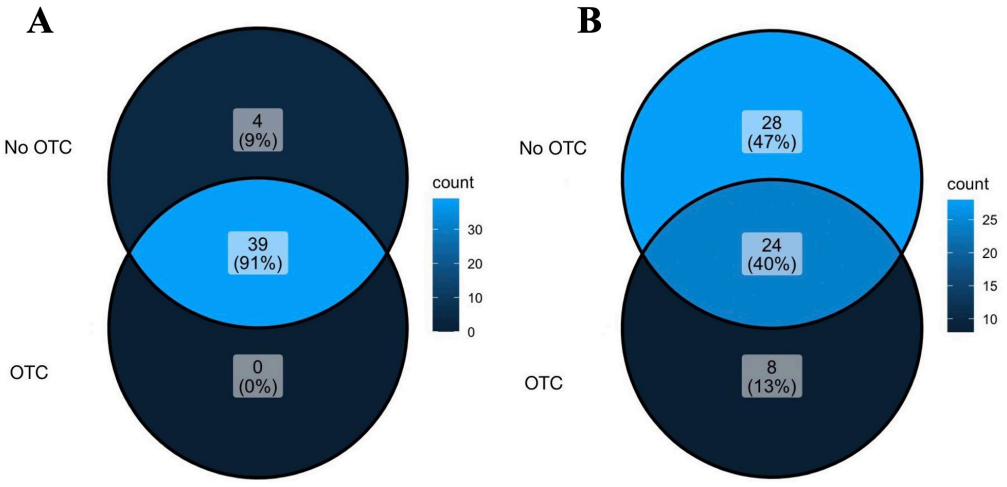

FIG. 4 OTC medication has a greater effect on microbial diversity in MS patients with eczema than patients without eczema. Core microbiome analysis was conducted on MS patients without (A) and with (B) eczema who do and do not take OTC medication. Analysis was completed with a detection threshold of 0.001 and a prevalence threshold of 0.5.

DISCUSSION

This study aimed to investigate potential associations of eczema with disease status and medication class in 20–50-year-old MS patients and healthy controls in order to elucidate the impact of eczema on the gut microbiome. We found that differences in community composition due to MS were only observed in patients without eczema (Figure 1). However, MS patients and healthy controls with eczema demonstrated similar levels of microbial community richness and abundance, as shown through alpha diversity analyses. Generally, some OTC medications are associated with changes in the gut microbiomes (21), but our study found that these changes in community composition were only prominent in MS patients without eczema (Figure 3). Thus, leading to the conclusion that eczema may have a

mediating effect on the gut microbiome, potentially masking the effects of MS and OTC medication usage on microbial diversity.

This study found that there were significant differences in community composition of the gut microbiome attributed to MS disease status only when eczema was not present as a comorbidity (Figure 1). While a plethora of literature indicates that MS changes the composition of the gut microbiome (7, 54–56), research on the impact of eczema on the gut microbiome remains relatively limited. Recent literature has only begun to explore the gut-skin axis and are yet to characterize their effects (57, 58). Additionally, much of the literature discusses the effects of a dysregulated gut microbiome in triggering skin diseases as opposed to the effect of skin diseases on the gut microbiome (59). For example, Kim *et al.* hypothesized that due to the more modern sanitized living conditions, a lack of microbial exposure in early life results in inadequate immune priming; thus, resulting in a predisposition to immune conditions such as eczema (58). However, one study by Watanabe *et al.* found that specifically *Bifidobacterium* was found to be significantly lower in patients with eczema (58). Thus, suggesting that the presence of eczema alters the composition of the gut microbiome. Our research aligns with this assertion as it suggests that the effects of eczema on the gut microbiome may be significant enough to mask the effects of MS on the gut microbiome (Figure 1).

The hypothesized masking effect observed by eczema on MS aligns with current literature surrounding eczema and MS; Lusignan *et al.* found that patients with eczema had changes in the gut microbiome that increased susceptibility to developing gastrointestinal conditions such as Crohn's disease and ulcerative colitis (60). This suggests that the changes associated with eczema in the gut microbiome are highly distinct. Our study results align with this assertion as the effects of MS on the gut microbiome community composition are only observed when eczema is not present.

Common OTC medications such as aspirin and decongestants have been implicated with altering the gut microbiome composition (61–63). Thus, our study explored whether these effects were prevalent in MS patients with and without eczema. The findings of the study support that OTC medication alterations on the gut microbiome are only prevalent when eczema is not present as a comorbidity (Figure 3). This suggests that eczema may mask the gut microbiome changes associated with OTC medication usage.

Many papers discuss the positive outcomes of OTC usage in treating and managing eczema (64–66). This suggests that OTC medication could have a positive effect on the gut microbiomes of patients with eczema However, our results contradict this assertion which may be due to the specific type of OTC medication taken by each patient. For example, if patients were taking OTC medication that mainly treated their MS symptoms and not their eczema symptoms, this potentially could have resulted in the effect of eczema on community composition being greater. Additionally, patients identified as using OTC medication may not be consuming it frequently, thereby potentially limiting the overall effects of OTC medication compared to a persistent condition like eczema.

In contrast, MS patients without eczema exhibited a more similar core microbiome profile between individuals taking and not taking OTC medication than those with eczema (Figure 4). A working hypothesis to explain this contradiction is that disease states, namely MS and eczema, create a gut microbiome environment that is particularly susceptible to alterations in microbial composition induced by OTC medication usage. This connection is supported by literature that suggests that all three of these conditions (eczema, MS, and OTC medication usage) are associated with dysbiosis of the gut microbiome (12, 67, 68). Thus, potentially explaining why having both eczema and MS lead to a more distinct core microbiome when comparing OTC medication usage (Figure 4). However, because the core microbiome analysis appears to contradict other findings in this study, further investigation into the impact of each condition on the susceptibility of the gut microbiome to dysbiosis are needed to elucidate the relationship between MS, eczema, and OTC medication usage. Additionally, further characterization of the dysbiosis of the gut microbiome observed and the relative severity of dysbiosis may help elucidate whether eczema truly has a greater effect on the gut microbiome compared to OTC medication usage in MS patients.

Lastly, although beta diversity analysis revealed that there were differences in gut microbial composition attributed to OTC medication usage in MS patients without eczema,

overall microbial evenness and richness within groups, as elucidated by alpha diversity analysis, remained similar (Figure S4). This is reasonably in line with other literature that suggest that there are differences in the presence and ratio of some bacterial species, but no loss or gain of species (69). It is important to note that species found in the skin microbiome often differ greatly from the gut microbiome. Specifically, the presence of eczema typically leads to changes in microbial diversity, notably with increases in abundance of *Staphylococcus aureus* (70). Thus, further research that better characterises gut microbiome changes in patients with eczema would help to elucidate why evenness and richness within groups does not appear to change drastically.

In line with previous literature, this study identified specific indicator taxa that are associated with eczema and OTC medication usage (Table 1). However, contrary to previous findings, the specific taxa identified in this study differed (71). Previous literature linked eczema with microbial taxa such as *Escherichia, Clostridium, Bacteroidota, Bifidobacterium,* and *Akkermansia* (71); this study also found a strong association between eczema and *Bacteroidota,* but no associations with the other taxa (Table 1). Given that *Bacteroidota* species were identified as a strong indicator of eczema (Table 1), alterations in these taxa may serve as a marker for eczema status. It's worth noting that *Bacteroidota* species are abundant in the human gastrointestinal tract and are generally considered symbiotic (72–75). However, the relevance of this bacterial phylum to eczema status has not been extensively studied. Further characterization of the mechanisms of action and function of *Bacteroidota* species is needed for a more robust understanding of their relationship with eczema.

Similarly, the use of OTC medication such as aspirin has been linked to decreases in *Bacteroidota* species present in the gut microbiome (61). This aligns with the findings of this study, an indicator taxa in this phylum was observed (Table 1). Numerous indicator taxa from the phylum *Actinobacteriota* were identified in MS patients taking OTC medication (Table 1). This phylum consists of microbes primarily associated with soil systems; however, certain genera are known to inhabit the human gut (76, 77). One such genus is *Corynebacterium*, generally benign in the gut microbiome but encompassing species that can be pathogenic and lead to adverse health effects (78–80). Therefore, *Actinobacteriota* species might serve as predictors for MS patients taking OTC medication, possibly attributed to the pathogenic nature of certain species. All in all, the presence of distinct indicator taxa in MS patients with eczema and using OTC medication, absent in those without eczema and not taking OTC medication, suggests that eczema and OTC usage results in a distinct change in the gut microbiome profile.

Limitations One major limitation of our study is the lack of information regarding the severity of eczema in patients. Since eczema is linked to dysbiosis of the gut microbiome, the degree of dysbiosis may change depending on the overall severity of eczema (57). Luger *et al.* discuss how eczema is commonly implicated with dysbiosis of the skin microbiome and further expound upon how this may transfer through the gut-skin axis and affect the gut microbiome (81). Since differing levels of changes in the skin microbiome can be seen with differing levels of severity of eczema, it is not too far a leap to presume that this correlates with dysbiosis of the gut microbiome (68). If we had information surrounding the severity of eczema, we may have found that only high severity of eczema would result in dysbiosis of the gut microbiome. Thus, not having information on the level of severity of the patients' eczema may confound our results.

Another limitation is that there is no information regarding the type of OTC medication taken by each patient. This is because some OTC medication is associated with strong changes in the gut microbiome, such as aspirin and decongestants, while others are less associated with changes in the gut microbiome (61, 63). Chung *et al.* found that the use of decongestants strongly alters the gut microbiome and results in dysbiosis, whereas, there is no literature surrounding the alteration of the gut microbiome due to oral acetaminophen (63). Additionally, Bai *et al.* found that aspirin results in a change in the gut microbiome, specifically an increase in the phylum Bacteroidetes and a decrease in the ratio of Firmicutes to Bacteroidetes (63). Since there is potential that the changes in microbial community composition due to each of these different OTC medications can be drastically different depending on the type of OTC medication taken, this potentially confounds our results.

Additionally, limitations arise from the decreasing sample size with respect to increasing specific subsets. Specifically, there were only two individuals each who had MS, were not using prescription medication, and were taking or not taking OTC medication (Table S1). The small sample size may fail to accurately represent the broader population. Additionally, to conserve samples, confounding factors such as sex and BMI were not accounted for. Lastly, the dataset used in this study represents relative abundance information, as opposed to having total abundance data. These factors could also potentially influence the findings and interpretations of this study.

Conclusions Our study investigated the potential effects of eczema on the gut microbiomes of patients with MS and those taking varying medication classes. The study found that eczema may have a mediating effect that masks the changes in the gut microbiome caused by MS and OTC medication. This was demonstrated through microbial changes due to MS only being visible when eczema was not present as a comorbidity. Similarly, the effects of OTC medication on the gut microbiome composition were only perceivable in MS patients when eczema was not present. The implications of these findings are that potentially MS patients with eczema have a unique gut microbiome composition that is dissimilar to MS patients without eczema and that this should be considered in treatment options. However, further studies should analyse whether this difference is significant in clinical settings and affects the efficacy of current treatments on MS progression. Better characterization of unique microbiomes in MS patients can help inform treatment courses.

Future Directions In future studies, the magnitude of dysbiosis due to MS, eczema, and OTC medication use should be better characterised in comparison to one another. This would help to elucidate which condition may result in a more profound dysbiosis of the gut microbiome as compared to healthy controls and the contrast between our beta diversity results that suggest that eczema is the most influential factor and the core microbiome that suggests the opposite. This could be done through an *in vivo* study that compares mice with MS, eczema, and two or three different OTC medications compared to healthy controls. Differential gene expression analysis using DESeq2 of genes involved in the inflammasome and metabolism could better characterize the differential abundance between these conditions.

Further research surrounding the specific mechanisms of mediation seen in MS patients with eczema and their clinical relevance should be further explored. This could be done through exploring the mechanisms of action of the unique species seen in eczema. By first isolating and characterizing these species, predictions of the mechanisms of action that may impact disease and eczema states can be made. Followed by a metabolomic analysis and *in vitro* assay, the function of the species of interest can be characterized. This characterization could then be tested in animal models to test the hypothesized mechanism of action in the diseased state.

Another analysis that would further the findings in this study are to separate the analyses of different types of OTC medication. This would help to parse whether particular OTC medications have a significant effect on the gut microbiome without being confounded by other types of OTC medication. This is because different types of OTC medication have been implicated with different changes in the gut microbiome; thus, likely resulting in different profiles of the gut microbiome in terms of composition and diversity (63). Additionally, frequency of use should be taken into consideration as for certain types of OTC medication dysbiosis is only observed at high levels of use (82).

DATA AVAILABILITY

Scripts used in QIIME2 and R/RStudio analyses are available at https://github.com/clarekonnert/MICB475-Team-1

ACKNOWLEDGEMENTS

We extend our gratitude to Dr. Evelyn Sun, Bessie Wang, and the entire MICB 475 teaching team for their invaluable support and guidance during the course of this project. We are also thankful to the UBC Department of Microbiology and Immunology for its funding and

provision of resources. Furthermore, we would like to acknowledge the iMSMS Consortium for graciously providing the dataset utilised in this project. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

Kaitlin Law (KL) and Anny Xia (AX) led the initial data wrangling process, with KL focusing on metadata manipulation and AX performing the dataset processing using QIIME2. Clare Konnert (CK) and Michelle Tong (MT) wrote the scripts and conducted beta and alpha diversity analyses respectively, using R/RStudio. KL was responsible for scripting and executing the core microbiome and indicator taxa species analyses. KL, AX, CK, MT and Davey Li (DL) contributed equally to manuscript preparation, including drafting and editing the abstract, introduction, methods, results, discussion, limitations, conclusions, and future directions.

REFERENCES

1. **McGinley MP, Goldschmidt CH, Rae-Grant AD.** 2021. Diagnosis and Treatment of Multiple Sclerosis: A Review. *JAMA* **325:**765–779.
2. **Jakimovski D, Bittner S, Zivadinov R, Morrow SA, Benedict RH, Zipp F, Weinstock-Guttman B.** 2024. Multiple sclerosis. *The Lancet* **403**:183–202.
3. **Alfredsson L, Olsson T.** 2019. Lifestyle and Environmental Factors in Multiple Sclerosis. Cold Spring Harb Perspect *Med* **9**.
4. **Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, Yanortsang TB, Yang L, Jupp R, Mathis D, Benoist C, Kasper DL.** 2017. Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell* **168**:928-943.e11.
5. **Honda K, Littman DR.** 2016. The microbiota in adaptive immune homeostasis and disease. *Nature* **535**:75–84.
6. **Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE, Kümpfel T, Hohlfeld R, Krishnamoorthy G, Wekerle H.** 2017. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci* **114**:10719–10724.
7. **Correale J, Hohlfeld R, Baranzini SE. 2022**. The role of the gut microbiota in multiple sclerosis. *Nat Rev Neurol* **18**:544–558.
8. **Kadowaki A, Quintana FJ.** 2020. The Gut-CNS Axis in Multiple Sclerosis. *Trends Neurosci* **43**:622–634.
9. **Pröbstel A-K, Baranzini SE.** 2018. The Role of the Gut Microbiome in Multiple Sclerosis Risk and Progression: Towards Characterization of the "MS Microbiome." *Neurotherapeutics* **15**:126–134.
10. **Chen J, Chia N, Kalari KR, Yao JZ, Novotna M, Paz Soldan MM, Luckey DH, Marietta EV, Jeraldo PR, Chen X, Weinshenker BG, Rodriguez M, Kantarci OH, Nelson H, Murray JA, Mangalam AK.** 2016. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* **6**:28484.
11. **Camara-Lemarroy CR, Metz LM, Yong VW.** 2018. Focus on the gut-brain axis: Multiple sclerosis, the intestinal barrier and the microbiome. *World J Gastroenterol* **24**:4217–4223.
12. **Cox LM, Maghzi AH, Liu S, Tankou SK, Dhang FH, Willocq V, Song A, Wasén C, Tauhid S, Chu R, Anderson MC, De Jager PL, Polgar-Turcsanyi M, Healy BC, Glanz BI, Bakshi R, Chitnis T, Weiner HL.** 2021. Gut Microbiome in Progressive Multiple Sclerosis. *Ann Neurol* **89**:1195–1211.
13. **Zhou X, Baumann R, Gao X, Mendoza M, Singh S, Sand IK, Xia Z, Cox LM, Chitnis T, Yoon H, Moles L, Caillier SJ, Santaniello A, Ackermann G, Harroud A, Lincoln R, Gomez R, Peña AG, Digga E, Hakim DJ, Vazquez-Baeza Y, Soman K, Warto S, Humphrey G, Farez M, Gerdes LA, Oksenberg JR, Zamvil SS, Chandran S, Connick P, Otaegui D, Castillo-Triviño T, Hauser SL, Gelfand JM, Weiner HL, Hohlfeld R, Wekerle H, Graves J, Bar-Or A, Cree BAC, Correale J, Knight R, Baranzini SE**. 2022. Gut microbiome of multiple sclerosis patients and paired household healthy controls reveal associations with disease risk and course. *Cell* **185**:3467-3486.e16.
14. **Eyerich K, Novak N.** 2013. Immunology of atopic eczema: overcoming the Th1/Th2 paradigm. *Allergy* **68**:974–982.
15. **Saferding V, Blüml S.** 2020. Innate immunity as the trigger of systemic autoimmune diseases. J *Autoimmun* **110**:102382.
16. **Lu Z, Zeng N, Cheng Y, Chen Y, Li Y, Lu Q, Xia Q, Luo D.** 2021. Atopic dermatitis and risk of autoimmune diseases: a systematic review and meta-analysis. *Allergy Asthma Clin Immunol* **17**:96.
17. **Andersen YMF, Egeberg A, Gislason GH, Skov L, Thyssen JP.** 2017. Autoimmune diseases in adults with atopic dermatitis. *J Am Acad Dermatol* **76**:274-280.e1.
18. **Zheng H, Liang H, Wang Y, Miao M, Shi T, Yang F, Liu E, Yuan W, Ji Z-S, Li D-K.** 2016. Altered Gut Microbiota Composition Associated with Eczema in Infants. *PLOS ONE* **11**:e0166026.
19. **Bieber K, Hundt JE, Yu X, Ehlers M, Petersen F, Karsten CM, Köhl J, Kridin K, Kalies K, Kasprick A, Goletz S, Humrich JY, Manz RA, Künstner A, Hammers CM, Akbarzadeh R, Busch H, Sadik CD, Lange T, Grasshoff H, Hackel AM, Erdmann J, König I, Raasch W, Becker M, Kerstein-Stähle A, Lamprecht P, Riemekasten G, Schmidt E, Ludwig RJ.** 2023. Autoimmune pre-disease. *Autoimmun Rev* **22**:103236.

20. **Eyerich K, Eyerich S, Biedermann T.** 2015. The Multi-Modal Immune Pathogenesis of Atopic Eczema. *Trends Immunol* **36**:788–801.
21. **Vich Vila A, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, Mujagic Z, Jonkers DMAE, Masclee AAM, Fu J, Kurilshikov A, Wijmenga C, Zhernakova A, Weersma RK.** 2020. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. *Nat Commun* **11**:362.
22. **Akdis CA.** 2021. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat Rev Immunol* **21**:739–751.
23. **Rogers MAM, Aronoff DM.** 2016. The Influence of Nonsteroidal Anti-Inflammatory Drugs on the Gut Microbiome. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis* **22**:178.e1-178.e9.
24. **Esposito M, De Berardinis A, Totaro R, Fargnoli MC.** 2023. Dupilumab in patients with moderate to severe atopic dermatitis and multiple sclerosis. *JAAD Case Rep* **31**:1–5.
25. **Brown SJ.** 2016. Atopic eczema. *Clin Med* **16**:66–69.
26. **Nolan BV, Levender MM, Davis SA, Feneran AN, Jr ABF, Feldman SR.** 2012. Trends in the use of topical over the counter products in the management of dermatologic disease in the United States. *Dermatol Online J* **18**.
27. **Lee JH, Son SW, Cho SH.** 2016. A Comprehensive Review of the Treatment of Atopic Eczema. *Allergy Asthma Immunol Res* **8**:181–190.
28. **Pilotto S, Zoledziewska M, Fenu G, Cocco E, Lorefice L.** 2023. Disease-modifying therapy for multiple sclerosis: Implications for gut microbiota. *Mult Scler Relat Disord* **73**.
29. **Ordoñez-Rodriguez A, Roman P, Rueda-Ruzafa L, Campos-Rios A, Cardona D.** 2023. Changes in Gut Microbiota and Multiple Sclerosis: A Systematic Review. *Int J Environ Res Public* Health **20**:4624.
30. **Del Negro I, Pez S, Versace S, Marziali A, Gigli GL, Tereshko Y, Valente M.** 2024. Impact of Disease-Modifying Therapies on Gut–Brain Axis in Multiple Sclerosis. *Medicina (Mex)* **60**:6.
31. **Thelen J, Zvonarev V, Lam S, Burkhardt C, Lynch S, Bruce J.** 2021. Polypharmacy in Multiple Sclerosis: Current Knowledge and Future Directions. *Mo Med* **118**:239–245.
32. **Chertcoff A, Ng HS, Zhu F, Zhao Y, Tremlett H.** 2023. Polypharmacy and multiple sclerosis: A population-based study. *Mult Scler J* **29**:107–118.
33. **Cookson W.** 2004. The immunogenetics of asthma and eczema: a new focus on the epithelium. 12. *Nat Rev Immunol* **4**:978–988.
34. Houari A, Ecale F, Mercier A, Crapart S, Laparre J, Soulard B, Ramnath M, Berjeaud J, Rodier M, Crépin A. 2022. Development of an in vitro model of human gut microbiota for screening the reciprocal interactions with antibiotics, drugs, and xenobiotics. Front Microbiol. 2022;13:828359.El
35. **Jangi S, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Liu S, Glanz BL, Cook S, Tankou S, Stuart F, Melo K, Nejad P, Smith K, Topçuolu BD, Holden J, Kivisäkk P, Chitnis T, De Jager PL, Quintana FJ, Gerber GK, Bry L, Weiner HL.** 2016. Alterations of the human gut microbiome in multiple sclerosis. *Nat Commun* **7**:12015.
36. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG.** 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**:852–857.
37. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP.** 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**:581–583.
38. **Silva**. https://www.arb-silva.de/. Retrieved 15 April 2024.
39. **Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO.** 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**:D590–D596.
40. **Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, Schweer T, Peplies J, Ludwig W, Glöckner FO.** 2014. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res* **42**:D643–D648.
41. 16S Illumina Amplicon Protocol : earthmicrobiome. https://earthmicrobiome.org/protocols-and-standards/16s/. Retrieved 15 April 2024.
42. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* **8**:e61217.
43. Who Gets MS? Natl Mult Scler Soc. https://www.nationalmssociety.org/What-is-MS/Who-Gets-MS. Retrieved 15 April 2024.

44. **Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H.** 2019. Welcome to the Tidyverse. *J Open Source Softw* **4**:1686.
45. **Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, Caceres MD, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette M-H, Cunha ER, Smith T, Stier A, Braak CJFT, Weedon J.** 2022. vegan: Community Ecology Package (2.6-4).
46. **Paradis E, Claude J, Strimmer K.** 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* **20**:289–290.
47. **Wickham H.** 2009. ggplot2: Elegant Graphics for Data Analysis. Springer, New York, NY. https://link.springer.com/10.1007/978-0-387-98141-3.
48. microbiome. Bioconductor. http://bioconductor.org/packages/microbiome/. Retrieved 15 April 2024.
49. **Yan L.** 2023. ggvenn: Draw Venn Diagram by "ggplot2" (0.1.10).
50. **Neuwirth E.** 2022. RColorBrewer: ColorBrewer Palettes (1.1-3).
51. **Wickham H.** 2007. Reshaping Data with the reshape Package. J Stat Softw *21*:**1**–20.
52. **Xie Y, Sarma A, Vogt A, Andrew A, Zvoleff A, Al-Zubaidi A, http://www.andre-simon.de) AS (the C files under inst/themes/ were derived from the H package, Atkins A, Wolen A, Manton A, Yasumoto A, Baumer B, Diggs B, Zhang B, Yapparov B, Pereira C, Dervieux C, Hall D, Hugh-Jones D, Robinson D, Hemken D, Murdoch D, Campitelli E, Hughes E, Riederer E, Hirschmann F, Simeon F, Fang F, inst/misc/Sweavel.sty) FEHJ (the S package at, Aden-Buie G, Detrez G, Wickham H, Zhu H, Jeon H, Bengtsson H, Yutani H, Lyttle I, Daniel H, Bien J, Burkhead J, Manton J, Lander J, Punyon J, Luraschi J, Arnold J, Bryan J, inst/misc/docco-classic.css) JA (the C file at, Stephens J, Hester J, Cheng J, Ranke J, Honaker J, Muschelli J, Keane J, Allaire JJ, Toloe J, Sidi J, Larmarange J, Barnier J, Zhong K, Slowikowski K, Forner K, Smith KK, Mueller K, Takahashi K, Walthert L, Gallindo L, Hofert M, Modrák M, Chirico M, Friendly M, Bojanowski M, Kuhlmann M, Patrick M, Caballero N, Salkowski N, Hansen NR, Ross N, Mahdi O, Krivitsky PN, Faria P, Li Q, Vaidyanathan R, Cotton R, Krzyzanowski R, Copetti R, Francois R, Williamson R, Mati S, Kostyshak S, Meyer S, Brouwer S, Bernard S de, Rousseau S, Wei T, Assus T, Lamadon T, Leeper T, Mastny T, Torsney-Weir T, Davis T, Veitas V, Zhu W, Wu W, Foster Z, Kamvar ZN, Software P, PBC.** 2024. knitr: A General-Purpose Package for Dynamic Report Generation in R (1.46).
53. **Cáceres MD, Legendre P.** 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**:3566–3574.
54. **Mou Y, Du Y, Zhou L, Yue J, Hu X, Liu Y, Chen S, Lin X, Zhang G, Xiao H, Dong B.** 2022. Gut Microbiota Interact With the Brain Through Systemic Chronic Inflammation: Implications on Neuroinflammation, Neurodegeneration, and Aging. *Front Immunol* **13**.
55. **Jayasinghe M, Prathiraja O, Kayani AMA, Jena R, Caldera D, Silva MS, Singhal M, Jr JP, Jayasinghe M, Prathiraja O, Kayani AMA, Jena R, Caldera D, Silva MS, Singhal M, Jr JAC-P.** 2022. The Role of Diet and Gut Microbiome in Multiple Sclerosis. *Cureus* **14**.
56. **Dunalska A, Saramak K, Szejko N.** 2023. The Role of Gut Microbiome in the Pathogenesis of Multiple Sclerosis and Related Disorders. *Cells* **12**:1760.
57. **Mahmud MdR, Akter S, Tamanna SK, Mazumder L, Esti IZ, Banerjee S, Akter S, Hasan MdR, Acharjee M, Hossain MdS, Pirttilä AM.** 2022. Impact of gut microbiome on skin health: gut-skin axis observed through the lenses of therapeutics and skin diseases. *Gut Microbes* **14**:2096995.
58. **Kim JE, Kim HS.** 2019. Microbiome of the Skin and Gut in Atopic Dermatitis (AD): Understanding the Pathophysiology and Finding Novel Management Strategies. *J Clin Med* **8**:444.
59. **Fang Z, Li L, Zhang H, Zhao J, Lu W, Chen W.** 2021. Gut Microbiota, Probiotics, and Their Interactions in Prevention and Treatment of Atopic Dermatitis: A Review. *Front Immunol* **12**.
60. **Lusignan S de, Alexander H, Broderick C, Dennis J, McGovern A, Feeney C, Flohr C.** 2022. Atopic dermatitis and risk of autoimmune conditions: Population-based cohort study. *J Allergy Clin Immunol* **150**:709–713.
61. **Bai Z, Liu Y, Zhao Y, Yan R, Yang L, Ma H, Wang J, Wang T, Li Y, Zhang G, Zhang X, Jia S, Wang H.** 2023. Aspirin ameliorates atherosclerotic immuno-inflammation through regulating the Treg/Th17 axis and CD39-CD73 adenosine signaling via remodeling the gut microbiota in ApoE-/- mice. *Int Immunopharmacol* **120**:110296.
62. **Liu KY, Wang Q, Nakatsu CH, Jones-Hall Y, Jiang Q.** 2023. Combining gamma-tocopherol and aspirin synergistically suppresses colitis-associated colon tumorigenesis and modulates the gut microbiota in mice, and inhibits the growth of human colon cancer cells. *Eur J Pharmacol* **946**:175656.
63. **Chung S-Y, Kostev K, Tanislav C.** 2023. Decongestants, proton pump inhibitors, and systemic antibiotics are associated with an increased occurrence of dysbiosis. *Int J Clin Pharmacol Ther* **61**:59–66.
64. **Kulthanan K, Tuchinda P, Nitiyarom R, Chunharas A, Chantaphakul H, Aunhachoke K, Chularojanamontri L, Rajatanavin N, Jirapongsananuruk O, Vichyanond P, Chatchatee P, Sangsupawanich P, Wananukul S, Singalavanija S, Trakanwittayarak S, Rerkpattanapipat T, Thongngarm T, Wisuthsarewong W, Limpongsanurak W, Kamchaisatian W, Noppakun N.** 2021. Clinical practice guidelines for the diagnosis and management of atopic dermatitis. *Asian Pac J Allergy Immunol* **39**:145–155.

65. Individual Article: Evaluation of Efficacy and Tolerability of Two Over-the-Counter Eczema Itch Relief Products. JDDonline - J Drugs Dermatol. https://jddonline.com/articles/evaluation-of-efficacy-and-tolerability-of-two-over-the-counter-eczema-itch-relief-products-S1545961623PSF388641s10X/. Retrieved 15 April 2024.
66. Individual Article: A Novel 3-Step Over-the-Counter Eczema Regimen Improves Eczema Severity, Itch, and Life Quality: Randomized Study. JDDonline - J Drugs Dermatol. https://jddonline.com/articles/a-novel-3-step-over-the-counter-eczema-regimen-improves-eczema-severity-itch-and-life-quality-randomized-study-S1545961623PSF388641s21X/. Retrieved 15 April 2024.
67. **Bicknell B, Liebert A, Borody T, Herkes G, McLachlan C, Kiat H.** 2023. Neurodegenerative and Neurodevelopmental Diseases and the Gut-Brain Axis: The Potential of Therapeutic Targeting of the Microbiome. *Int J Mol Sci* **24**:9577.
68. **Paller AS, Kong HH, Seed P, Naik S, Scharschmidt TC, Gallo RL, Luger T, Irvine AD.** 2019. The microbiome in patients with atopic dermatitis. *J Allergy Clin Immunol* **143**:26–35.
69. **Watanabe S, Narisawa Y, Arase S, Okamatsu H, Ikenaga T, Tajiri Y, Kumemura M.** 2003. Differences in fecal microflora between patients with atopic dermatitis and healthy control subjects. *J Allergy Clin Immunol* **111**:587–591.
70. **Edslev SM, Agner T, Andersen PS.** 2020. Skin Microbiome in Atopic Dermatitis. *Acta Derm Venereol* **100**:358–366.
71. **De Pessemier B, Grine L, Debaere M, Maes A, Paetzold B, Callewaert C.** 2021. Gut–Skin Axis: Current Knowledge of the Interrelationship between Microbial Dysbiosis and Skin Conditions. *Microorganisms* **9**:353.
72. **Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN.** 2015. Role of the normal gut microbiota. *World J Gastroenterol* **21**:8787–8803.
73. **Mariat D, Firmesse O, Levenez F, Guimarães V, Sokol H, Doré J, Corthier G, Furet J-P.** 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol* **9**:123.
74. **Parker BJ, Wearsch PA, Veloo ACM, Rodriguez-Palacios A.** 2020. The Genus Alistipes: Gut Bacteria With Emerging Implications to Inflammation, Cancer, and Mental Health. *Front Immunol* **11**.
75. **Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA.** 2005. Diversity of the Human Intestinal Microbial *Flora. Science* **308**:1635–1638.
76. **Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, Kerr C, Hourihane J, Murray D, Fuligni F, Gueimonde M, Margolles A, Bellis GD, O'Toole PW, Sinderen D van, Marchesi JR, Ventura M.** 2012. Diversity of Bifidobacteria within the Infant Gut Microbiota. *PLOS ONE* **7**:e36957.
77. **Servin JA, Herbold CW, Skophammer RG, Lake JA.** 2008. Evidence Excluding the Root of the Tree of Life from the Actinobacteria. *Mol Biol Evol* **25**:1–4.
78. **Rajamani Sekar SK, Veeraraghavan B, Anandan S, Devanga Ragupathi NK, Sangal L, Joshi S.** 2017. Strengthening the laboratory diagnosis of pathogenic Corynebacterium species in the Vaccine era. *Lett Appl Microbiol* **65**:354–365.
79. **Marosevic DV, Berger A, Kahlmeter G, Payer SK, Hörmansdorfer S, Sing A.** 2020. Antimicrobial susceptibility of Corynebacterium diphtheriae and Corynebacterium ulcerans in Germany 2011-17. *J Antimicrob Chemother* **75**:2885–2893.
80. **Li G, Wang S, Zhao S, Zhou Y, Pan X.** 2021. Whole genome sequence of a non-toxigenic Corynebacterium diphtheriae strain from a hospital in southeastern China. *BMC Genomic Data* **22**:42.
81. **Luger T, Amagai M, Dreno B, Dagnelie M-A, Liao W, Kabashima K, Schikowski T, Proksch E, Elias PM, Simon M, Simpson E, Grinich E, Schmuth M.** 2021. Atopic dermatitis: Role of the skin barrier, environment, microbiome, and therapeutic agents. *J Dermatol Sci* **102**:142–157.
82. **Hodkovicova N, Hollerova A, Blahova J, Mikula P, Crhanova M, Karasova D, Franc A, Pavlokova S, Mares J, Postulkova E, Tichy F, Marsalek P, Lanikova J, Faldyna M, Svobodova Z.** 2022. Non-steroidal anti-inflammatory drugs caused an outbreak of inflammation and oxidative stress with changes in the gut microbiota in rainbow trout (Oncorhynchus mykiss). *Sci Total Environ* **849**:157921.