Revista Argentina de Microbiología 57 (2025) 275–287

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

Microbial diversity investigation using 16S metagenomics in Tunisian patients with systemic lupus erythematosus

Amira Messadi[a,*], **Sameh Sayhi**[a,b,c], **Kais Ghedira**[d], **Chadia Zaouaoui**[e], **Bilel Arfaoui**[a,b,c], **Soumaya Khouikhi**[f], **Maha Rebai**[a,b,c], **Nour El Houda Guediche**[a,b,c], **Nadia Abdelhafidh**[a,b,c], **Bassem Louzir**[a,b,c], **Ajili Faida**[a,b,c]

[a] *Research Unit of Autoimmune Disease UR17DN02, Military Hospital of Tunis, Tunis, Tunisia*
[b] *Internal Medicine Department, Military Hospital of Tunis, Tunisia*
[c] *University Tunis EL Manar, Tunis, Tunisia*
[d] *Laboratory of Bioinformatics, Biomathematics and Biostatistics LR20IPT09, Pasteur Institute of Tunis, University of Tunis El Manar, Tunis, Tunisia*
[e] *Endocrinology Department, Military Hospital of Tunis, Tunis, Tunisia*
[f] *LR Biotechnology and Bio-Geo Resources Valorization (LR11ES31), University of Manouba, Tunisia*

Received 5 January 2025; accepted 4 April 2025
Available online 3 June 2025

KEYWORDS
Systemic lupus erythematous;
Microbiome diversity;
16S metagenomics;
Case–control studies;
Bioinformatics

Abstract Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease associated with significant morbidity and mortality. It is characterized by a loss of self-immune tolerance and autoantibody production, leading to multiple organ damage. Emerging investigations have confirmed the role of gut microbiota dysbiosis in patients with SLE, although the underlying mechanisms remain unclear to date. In this study, we aim to investigate the bacterial profile of SLE including phylum/class/genus relative abundance and diversity, to compare them with healthy controls and to study the correlation of relative abundance of different patterns with clinical/biological parameters. In this case–control study, the bacterial profile was investigated in 7 SLE patients and 7 healthy controls using 16S metagenomics clustering. The present study reported a low abundance of the class Bacilli (0.58% in SLE vs 1.26% in the controls), the genus *Lactobacillus* (0.43% vs 0.74%), as well as a higher abundance of the genera *Gammaproteobacteria* (2.37% vs 0.77%) and *Escherichia–Shigella* (2.04% vs 0.51%) in SLE samples compared to the controls ($p < 0.05$). We also found an association between the class Betaproteobacteria (4.42% vs 1.57%) and the genus *Faecalibacterium* (11.34% vs 3.35%) and

* Corresponding author.
E-mail address: amiramessadi@gmail.com (A. Messadi).

https://doi.org/10.1016/j.ram.2025.04.002

renal manifestations ($p < 0.05$). The phylum Actinobacteria (0.21% vs 3.8%, $p = 0.036$) and the genus *Bifidobacterium* levels were lower in active SLE compared to the healthy controls. This study is the first report on the gut microbiota of SLE and the first case–control study in Tunisia and North Africa. We obtained a particular profile of bacterial gut microbiota for the SLE group. We found a specific clustering when compared to the healthy controls.

PALABRAS CLAVE
Lupus eritematoso sistémico;
Diversidad del microbioma;
Metagenómica 16S;
Estudios de casos y controles;
Bioinformática

Investigación de la diversidad microbiana mediante metagenómica 16S en pacientes tunecinos con lupus eritematoso sistémico

Resumen El lupus eritematoso sistémico (LES) es una enfermedad autoinmune multisistémica que ocasiona elevada mortalidad y morbilidad. Se caracteriza por la pérdida de la tolerancia autoinmune y la producción de autoanticuerpos, que dañan múltiples órganos. En este trabajo, investigamos el perfil bacteriano del LES determinando las abundancias relativas a nivel de filo/clase/género y la diversidad en 7 pacientes con LES y 7 controles sanos, empleando agrupamiento metagenómico del gen 16S. Además, estudiamos la correlación de la abundancia relativa de diferentes *clusters* con parámetros clínicos/biológicos. En los pacientes con LES, encontramos baja abundancia relativa de la clase *Bacilli* (0,58% vs. 1,26% en controles) y del género *Lactobacillus* (0,43% vs. 0,74%), así como una mayor abundancia relativa de la clase *Gammaproteobacteria* (2,37% vs. 0,77%) y de los géneros *Escherichia-Shigella* (2,04% vs. 0,51%), en comparación con los controles ($p < 0,05$). También encontramos en el LES asociación de la clase *Betaproteobacteria* (4,42% vs. 1,57%) y el género *Faecalibacterium* (11,34% vs. 3,35%) con la manifestación renal ($p < 0,05$). Los niveles del filo *Actinobacteria* y del género *Bifidobacterium* disminuyeron en el LES activo frente a los controles sanos (0,21% vs. 3,8% y 0,15% vs. 3,7%, $p = 0.036$, ambos). Este es el primer informe sobre la microbiota intestinal en personas con LES y el primer estudio de casos y controles en Túnez y el norte de África. Observamos un perfil particular de microbiota intestinal bacteriana en el grupo con LES y detectamos un agrupamiento específico al compararlo con el perfil de los controles sanos.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by immune system deregulation, leading to the over-activation of immune T and B cells, and the production of antibodies against nuclear and cytoplasmic antigens. SLE manifestations are associated with multiple autoantibodies, including anti-nuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA) antibodies, causing immune complex deposition leading to tissue lesions in multiple organs (skin, kidneys, joints, muscles, heart, among others)[44,48]. Lower levels of serum complement components C3 and C4 were also described.[24] Anti-Sm, anti-SSA and anti-SSB antibodies are specific to Sjögren's disease, which can be associated with SLE in some patients.

SLE mainly affects individuals between 15 and 40 years old and especially young women. There are no studies concerning the prevalence of SLE in North Africa, except in Tunisia. In Tunisia, the hospital prevalence varies from one study to another and is estimated between 0.1 and 0.3%[19,22,29]. Patients suffer from variable clinical manifestations, ranging from mild joint and skin involvement to life-threatening features. They mainly suffer from renal, rheumatological and cutaneous mucosal features. The clinical complexity of SLE and the lack of pathological features or tests pose a diagnostic challenge for the clinician.

The etiology of SLE is still unclear. Several recent studies have suggested that the modification of the gut microbial composition may be related to SLE manifestations[14–16,28,36,41,47]. Increased evidence indicates that intestinal dysbiosis is related to several autoimmune diseases such as type 1 diabetes[3,12], multiple sclerosis[9,18,34], rheumatoid arthritis[42], Crohn's disease/ulcerative colitis[20,45] and obesity[2].

The gut microbiota is a complex ecosystem that includes all unicellular organisms hosted in the gastrointestinal tract (GI), mainly bacteria but also viruses, fungi and archaea[43]. It is composed of 10^{14} microorganisms and includes about 2000 different bacterial species[26]. The composition of the intestinal microbiota is generally inferred from fecal samples and is characterized by the presence of five phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria. Firmicutes and Bacteroidetes represent the main dominant phyla and are faced with modifications

due to factors such as environment, age, genetics, diet and infection[13].

The precise mechanisms underlying gut microbiota dysbiosis in SLE remain elusive to date. Potential factors include molecular mimicry, compromised intestinal barrier function disrupting gut homeostasis, thereby facilitating the translocation of gut microbiota and their products (such as LPS from Gram-positive bacteria) into the systemic circulation. This process can trigger inflammation and tissue damage. Furthermore, it may involve the leakage of pathogens, toxins, and bacteria from the gut lumen into other organs, a phenomenon known as ''leaky gut.'' Other potential contributors include bacterial biofilms, specific intestinal pathogen infections, gender disparities related to sex hormones, autophagy of intestinal epithelial cells, extracellular vesicles, and microRNAs[37].

Dysbiosis in SLE involves Treg-Th17 trans-differentiation and lymphocyte over-activation occurring within the gut mucosa. This process initiates immune responses against commensal microbiota, where antigens from translocated bacteria may resemble host structures, triggering cross-reactivity that leads to autoantibody production and organ damage in SLE patients – a phenomenon known as molecular mimicry[27,40].

Recent investigations have increasingly highlighted the significance of gut microbiota dysbiosis in SLE, underscored by multiple studies[14–16,28,46,51]. These studies predominantly focused on American and Asian populations, revealing geographic variations in the composition of fecal microbial communities.

The aim of our study was to identify the gut bacterial profile of Tunisian subjects suffering from SLE, by evaluating the relative abundance and diversity of different clusters, to compare them with the healthy controls and to study the correlation between the relative abundance of different clusters with clinical/biological parameters. The investigation of the gut microbiota correlation with clinical/biological parameters of SLE may help clinicians to improve diagnosis and prescribe the right treatment of lupus and its complications. This study is particularly original because it is the first to analyze gut microbiota in Tunisian SLE patients as well as across Tunisia, North Africa, and the MENA region.

Materials and methods

Ethics statement

All subjects included in this experiment provided their written informed consent, and the protocol of this study was approved by the Ethics Committee of the Military Hospital of Tunis.

Study subjects

Seven women with SLE were recruited from the Internal Medicine Department of the Military Hospital of Tunis between 2020 and 2021 (mean age 48 ± 13.8 years). All of them met at least four of the American College of Rheumatology criteria for the diagnosis of SLE[5]. Patients were excluded from further study based on the following criteria: (1) Pregnancy or breast-feeding; (2) Current inflammatory or autoimmune disease; (3) Current malignancy other than skin; (4) Serious infection within 3 months with hospitalization; (5) Antibiotic treatment within the preceding three months.

The activity levels have been categorized based on SLE activity index (SLEDAI) scores: no activity (SLEDAI = 0), remission (SLEDAI = 1–5), moderate to very high activity (6–20). A score greater than 5 often leads to initiating or modifying therapy in over half of the cases (Aringer 2019). SLEDAI values were obtained from only six patients, ranging from 3 to 22, indicating remission or active disease (Table 1).

SLEDAI is a validated model of disease activity in lupus. Four of 7 patients had active disease at the time of sampling (SLEDAI > 5) and only 2 were in remission (SLEDAI $\leq$ 5).

The control group was composed of 7 healthy women recruited from the Military Hospital of Tunis (mean age 43.2 ± 12.2 years); they were age and sex-matched with the SLE patients, with no history of inflammatory/autoimmune diseases, nor any gastrointestinal tract infection, and had not received antibiotics within the preceding 3 months. Clinical laboratory tests were conducted as part of routine care. Patients and healthy controls were requested to provide fecal samples. Samples were conserved at $-80\,^{\circ}C$ at the Military Hospital of Tunis and then transferred to the African Biotechnology Society (ABS) laboratory for the next steps.

(i) *Anthropometric measurements*. Body mass index (BMI) was calculated using the following formula: weight/(height)^2 (in kg/m^2). Individuals with a BMI higher than 30 were excluded from the study given that the gut microbiota is influenced by obesity statuses[2]. Mean BMI value in SLE patients was 25.3 ± 3.8 and 23.8 ± 2.4 in the healthy controls (Table 1).

(ii) *Nutritional evaluation*. Participants were surveyed about their food consumption and evaluated as ''regular consumers'' or ''medium frequency consumers'' by the nutritionist. The food survey focused on beverage consumption, high-fiber foods such as fruits, vegetables, cereals, and dried fruits. Participants were asked about their intake of meats, oils and fat foods.

(iii) *Lifestyle-related factors*. Various lifestyle factors of the subjects were recorded. These factors encompassed smoking habits, physical activity, alcohol consumption and the use of supplements such as vitamins and minerals within the last month.

Immunologic assays

C3 and C4 levels and the presence or absence of autoantibodies including anti-nuclear antibodies (ANA), anti-dsDNA, anti-SSA, anti-SSB, anti-histone, anti-Sm, and anti-nucleosome, were recorded in SLE patients (Table 2). Normal values of C3 and C4 complement components were between 0.97–1.576 and 0.162–0.445, respectively.

Bacterial DNA extraction

DNA extraction and sequencing steps were performed in the ABS laboratory under sterilized and adequate conditions. A quantity of 180–220 mg of fecal samples were collected

Table 1 Demographic and clinical parameters in the two groups.

	Patients	Healthy controls	*p*-Value
Population	SLE patients	Healthy controls	
Place	Military Hospital of Tunis		
Period	2020–2021		
*Age (years)**	48 ± 13.8	43.2 ± 12.2	0.51
*BMI (kg/m²)**	25.3 ± 3.8	23.8 ± 2.4	0.40
*SLEDAI**	11 ± 7.1	–	
*Disease duration (years)**	15.4 ± 9.1	–	
Clinical manifestations, n (%)		–	
Rheumatological	3 (42.8)		
Renal	3 (42.8)		
Others**	3 (42.8)		
Treatment, n (%)		–	
Immunosuppressive	6 (85.7)		
Immunomodulator	5 (71.4)		
Immunotherapy	1 (14.3)		
Drug discontinuation	1 (14.3)		
Dietary intake, n (%)			
Fiber diet***	67.8%	75%	>0.05
Tea consumer	6 (85.7)	3 (42.8)	>0.05
Dairy products	4 (57.1)	7 (100)	>0.05

SLEDAI: SLE disease activity index, SLE: systemic lupus erythematous, BMI: bone mass index.
* Mean ± standard deviation.
** Others: cutaneous/mucosal, neurologic/neuropsychiatric and gastrointestinal symptoms with 14.3% for each manifestation.
*** Consumer proportion of high-fiber foods (fruits, vegetables, cereals and/or dried vegetables).

to perform bacterial DNA extraction from the study subjects using the QiAmp Fast DNA Stool Mini Kit (Qiagen) according to the manufacturer's guidelines. QiaXpert Spectrophotometer (Qiagen) was used to quantify DNA and 260/230 and 260/280 ratios were measured to check purity. DNA solutions were stored at −20 °C until being analyzed (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf, downloaded on 21-07-2024).

Preparation of the QIAseq 16S V3-V4 region panel PCR reaction

In this step, we mixed 1 μl of extracted bacterial DNA, 2.5 μl of UCP master mix, 1 μl of region panel and UCP PCR water for a volume of 10 μl per sample. The setup of QIAseq 16S Region PCR Reaction included a hold step at 95 °C for 2 min, 12 cycles with a 3-step cycling consisting of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s and extension at 72 °C for 2 min, and a final step at 72 °C for 7 min. The product was maintained at 4 °C. Then, we purified the amplified fragments using a magnetic reaction.

Preparation of the QIAseq 16S V3-V4 region panel sample index PCR reaction

To 32.5 μl of the QIAseq 16S region panel PCR product, we added 12.5 μl of UCP master mix, 2.5 μl of each primer (4 μM) and UCP PCR water to complete a volume of 50 μl per sample. The setup of QIAseq 16S Region PCR Reaction included a hold step at 95 °C for 2 min, 19 cycles with a 3-step cycling consisting of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 2 min, and a final step at 72 °C for 7 min. The product was maintained at 4 °C. We performed the same protocol of purification as the previous step and we stored the completed QIAseq 16S Region Panel Sequencing library at −20 °C.

Illumina MiSeq sequencing of 16S rRNA gene-based amplicons

To prepare for MiSeq sequencing, pooled libraries were denatured with NaOH, and then heat-denatured following a dilution in the hybridization buffer. For each sequencing run, at least 5% PhiX was included as an internal control. Sequencing was conducted on Illumina MiSeq technology using paired 300-bp reads and MiSeq v3 reagents. The overlapping ends of each read produce full-length reads of the V3 and V4 regions.

Bioinformatic and statistical analysis

The bioinformatic and statistical analysis was performed at the Pasteur Institute of Tunis and Military Hospital of Tunis. The FASTQ files were analyzed using mothur v.1.48.0 following the MiSeq Standard Operating Process pipeline (https://mothur.org/wiki/miseq_sop/). Operational Taxonomic Units (OTUs) were calculated at phylum, class and genus levels. Only taxa with relative abundance higher than 1% were considered for statistical analysis. Contigs were

Table 2 Presence of autoantibodies, C3/C4 and treatment in SLE patients.

Patients	Autoantibodies	C3	C4	Treatment	Immunosuppressive therapy
P1	P1. ANA (+), anti-dsDNA (−), anti-histone (−), anti-nucleosome (−)	1.550	0.161	Hydroxychloroquine, methotrexate, steroids	+
P2	P2. ANA (−)	**0.745**	0.217	Steroids, rituximab	+
P3	P3. ANA (+)	**0.172**	**0.064**	Hydroxychloroquine + steroids + MMF	+
P4	P4. ANA (+), anti-dsDNA (+), anti-histone (+), anti-nucelosome (+), anti-SSA (−), anti-SSB (−), anti-Sm (−)	–	–	Drug discontinuation	–
P5	ANA (+), anti-histone (−), anti-nucleosome (−), anti-SSA (+), anti-SSB (+), anti-Sm (−)	–	–	Hydroxychloroquine + steroids + MMF	+
P6	ANA (+), anti-histone (+), anti-nucleosome (+), anti-SSA (+), anti-SSB (+), anti-Sm (+)	1.550	0.217	Hydroxychloroquine + steroids + methotrexate	+
P7	ANA (+), anti-dsDNA (+), anti-histone (+), anti-nucleosome (+), anti-SSA (+), anti-SSB (−), anti-Sm (−)	1.120	**0.110**	Hydroxychloroquine + steroids	+

(+) positive, (−) negative, ANA: anti-nuclear antibody, MMF: mycophenolate mofetil, +: immunosuppressive therapy, values in bold: diminished levels of C3 or C4 complement component, anti-SSA: anti–Sjögren's-syndrome-related antigen A autoantibodies, anti-SSB: anti- Sjögren syndrome type B antigen autoantibodies.

checked to remove sequences that did not align with the SILVA V4 database[38]. For statistical analysis and data visualization, several R packages including phyloseq, ggplot2 and dplyr were used. Alpha-diversity estimators, corresponding to variables describing diversity within the samples (''Observed'', ''Chao1'', ''ACE'', ''Shannon'', ''Simpson'', ''InvSimpson'' diversity indexes), were computed. An ordination technique based on non-metric multidimensional scaling (NMDS) was used to explore microbiome data in the two groups (cases and controls). Beta-diversity analysis, including Jaccard and Bray–Curtis distance metrics[7,38], was then performed to check whether bacterial communities in the two groups were significantly different from each other. Statistical analysis was performed using SPSS 22.0 software. The normality of quantitative variables was evaluated and adequate parametric or non-parametric tests were used. A *T*-test was used to compare independent variables with a normal distribution and the Mann–Whitney test was used to compare independent variables to non-normally distributed ones. The chi-square test was performed to investigate the relationship between clinical manifestations and the relative abundance of different bacterial clusters. *p*-Values <0.05 were considered statistically significant.

Results

The demographic parameters of the participants and their clinical data such as age, BMI, disease duration, SLEDAI, clinical manifestations, treatment and dietary intake are shown in Table 1. The presence of autoantibodies, C3/C4 levels and treatment data of each patient are detailed in Table 2.

There were no significant differences between the patients and the healthy controls in terms of age, BMI, smoking history, and alcohol or dietary intake; none of them were smokers or alcohol consumers. The group of SLE patients included individuals with a wide variety of symptoms, mainly rheumatological (42.8%) or renal (42.8%). Of seven patients, five (71.4%) were treated with hydroxychloroquine as an immunomodulator, five received immunosuppressives such as steroids, methotrexate, or mycophenolate mofetil (MMF), one (14.3%) was treated with immunotherapy (Rituzimab) and one received no treatment (Tables 1 and 2).

Furthermore, the dietary intake proportions of fiber compounds, tea consumption, dairy products, proteins and lipids were recorded. There were no significant differences between the patients and the healthy controls (Table 1).

Six patients (85.7%) were positive for anti-nuclear antibodies (ANA) while only two (28.5%) patients were positive for anti-dsDNA antibodies. Two patients exhibited a decrease in C3 levels whereas three showed decreased levels of the C4 complement component (Table 2).

The metagenomics analysis of the samples showed a significant variation in the number of reads, ranging from 65 reads to 73 354 reads across the samples.

Gut microbiota profile in SLE patients and comparison with healthy controls:

The gut bacterial distribution of SLE patients and controls is illustrated in Figure 1. Healthy controls were numbered from 049 to 055, while SLE patients from 056 to 062 SLE patients. Bacteroidetes and Firmicutes were found to be the dominant phyla in all samples from both groups. These results are in line with previous studies, indicating that a healthy human gut microbiota is primarily composed of Bacteroidetes and Firmicutes[46]. The Firmicutes/Bacteroidetes ratio was 1.29 in SLE cases and 0.81 in the healthy controls. Moreover, Bacteroidetes and Firmicutes, Proteobacteria and Actinobacteria were present with different abundance levels across samples. However, no significant differences were observed at the phylum level. At the class level, we observed that *Bacilli*, belonging to the Firmicutes phylum, were less abundant in the SLE cases than in the healthy controls (0.58% vs 1.26%, $p < 0.05$). Furthermore, *Gammaproteobacteria*, belonging to the Proteobacteria phylum (2.37% vs 0.77%, $p < 0.05$) seemed to be more abundant in SLE patients compared to the controls. Finally, abundance at the genus level confirmed the microbial distribution observed at the class level, showing an increase in the abundance of Enterobacteriaceae, especially the genera *Escherichia* and *Shigella* (2.04% vs 0.51%, $p < 0.05$) in SLE samples compared to the controls. A decrease in the abundance of Lactobacillaceae, particularly *Lactobacillus* (0.43% vs 0.74%, $p < 0.05$) was observed within SLE samples compared to controls.

We estimated alpha diversity using the Observed_OTUs, Chao1, ACE, Shannon, Simpson, and InvSimpson alpha-diversity indices to compare the richness and evenness of the human gut microbiota between the two studied groups. The results of the alpha-diversity measures are highlighted in Figure 2. We observed that there was a difference in diversity between the case and the control samples.

Figure 3 displays the abundances of the top 100 OTUs for the case and control samples, ranked on the abundance for each group. The number of detected OTUs is generally significantly higher for the case samples compared to the control samples. In addition, it seemed that the evenness (Shannon) in the control and case samples were similar, indicating that the OTUs may be different in terms of abundance.

The beta-diversity analysis between the two groups of samples showed that the Jaccard distance did not provide a clear separation between cases and controls. Conversely, the Bray–Curtis distance provided a slightly better separation, showing that the two groups shared their abundant taxa but differed in their rare taxa. This suggests that although the groups had taxa in common, their specific taxa were different (Fig. 4).

Relationship between the relative abundance of bacterial clusters and clinical/biological parameters

No differences or correlations with the clinical features (SLEDAI and clinical manifestations), complement components or any antibodies were detected for phylum clustering ($p > 0.05$) [data not shown].

Sample classification revealed some specific clustering patterns in the patient samples, such as a significant difference between the relative abundance of class Betaproteobacteria (phylum Proteobacteria) (4.42% vs 1.57%) and genus *Faecalibacterium* (phylum Firmicutes, class Clostridia, family Ruminococcaceae) (11.34% vs 3.35%) and renal manifestations ($p = 0.042$, $p = 0.048$ respectively).

A negative correlation was detected between genus *Clostridium_XVIII* (phylum Firmicutes, class Erysipelotrichia, family Erysipelotrichaceae) and SLEDAI scores ($r = -0.812$, $p = 0.050$), whereas a positive correlation was found between genus *Odoribacter* genus (phylum Bacteroidetes, class Bacteroidia, family Porphyromonadaceae) and disease duration ($r = 0.924$, $p = 0.003$). The genus *Prevotella* (phylum Bacteroidetes, class Bacteroidia, family Prevotellaceae) seems to be negatively correlated with BMI index ($r = -0.757$, $p = 0.014$).

Moreover, we checked the relationship of gut microbiota abundance and diversity with the disease activity. All six patients with SLEDAI values were treated with immunosuppressive drugs.

We found lower levels of phylum/class Actinobacteria (0.21% vs 3.8%, $p = 0.036$) and genus *Bifidobacterium* (phylum Actinobacteria class Actinobacteria) (0.15% vs 3.7%, $p = 0.036$) in SLE patients with active disease compared to the healthy controls.

Discussion

SLE manifestations are linked to multiple autoantibodies, which facilitate immune complex formation and deposition, as well as other immune processes[44]. This intricate clinical presentation and pathogenesis make SLE a challenging disease to understand and define.

This study is the first report on the gut microbiota of SLE and first case–control study in Tunisia and North Africa. In the present study we performed a 16S metagenomics analysis to investigate the gut microbiota of Tunisian subjects, with and without SLE, through the evaluation of the proportions of Firmicutes, Bacteroidetes and other bacterial species. Indeed, the healthy controls recruited in the present study had no gastrointestinal tract disorders and had not received antibiotics within 3 months prior to sample collection. All patients were women, six of the seven individuals recruited were in the active and remission form of the disease at the time of sampling (SLEDAI score ranging from 3 to 22) and had received immunotherapy, immunosuppressive or immunomodulatory treatment in the previous months. These treatments have an impact on the physiology of SLE.

We found an altered microbiome in human SLE patients compared to the healthy controls. These results are in line with previous studies, indicating that a healthy human

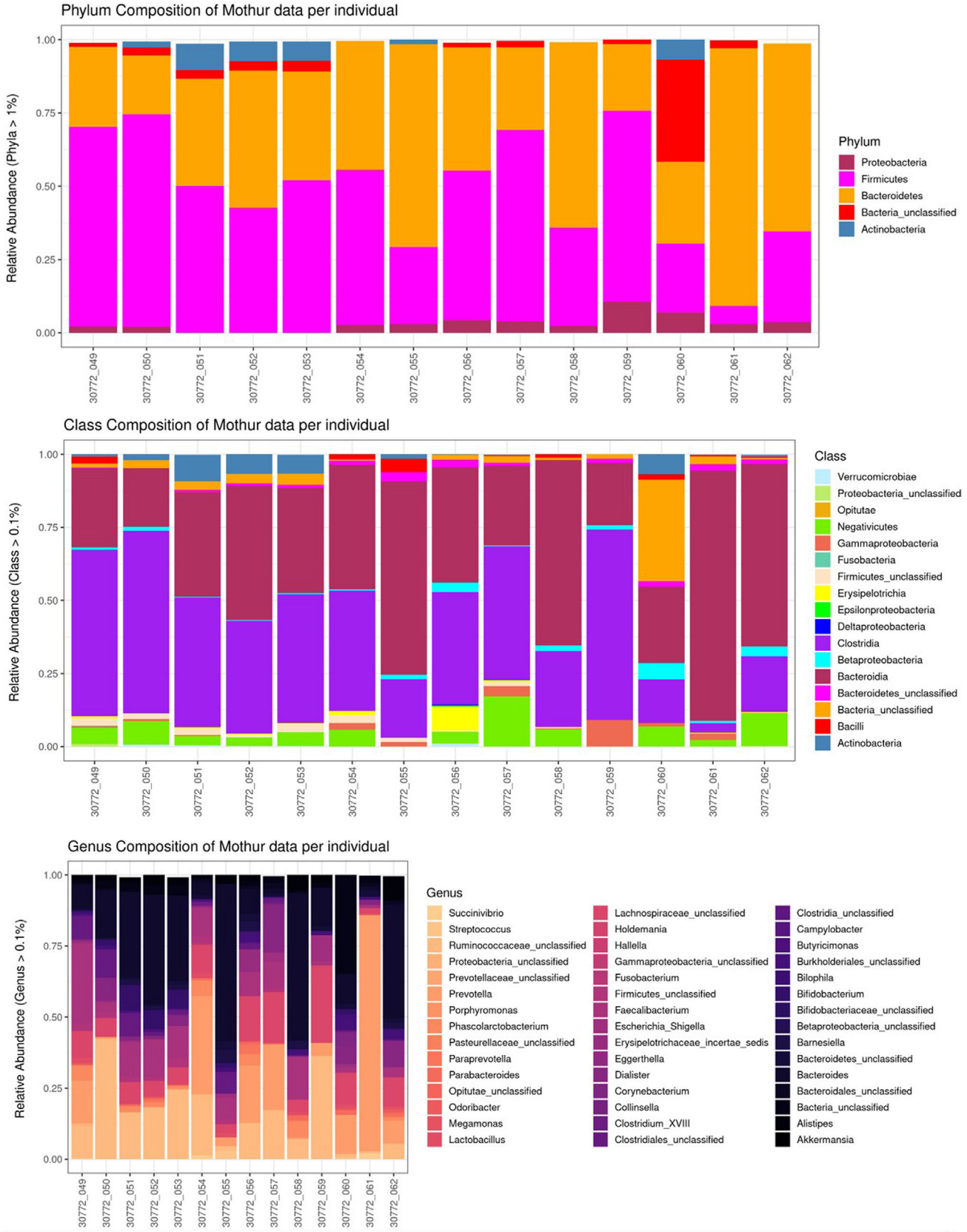

Figure 1 Relative abundance of the gut microbiota identified in our study within each control and SLE sample (049–055 numbers represent controls and 056–062 represent SLE patients). Relative abundance bar plots represent the bacterial composition in the human gut microbiota at the phylum, class, and genus levels, identified in each sample. Each legend box shows the top classified bacterial taxa across the samples.

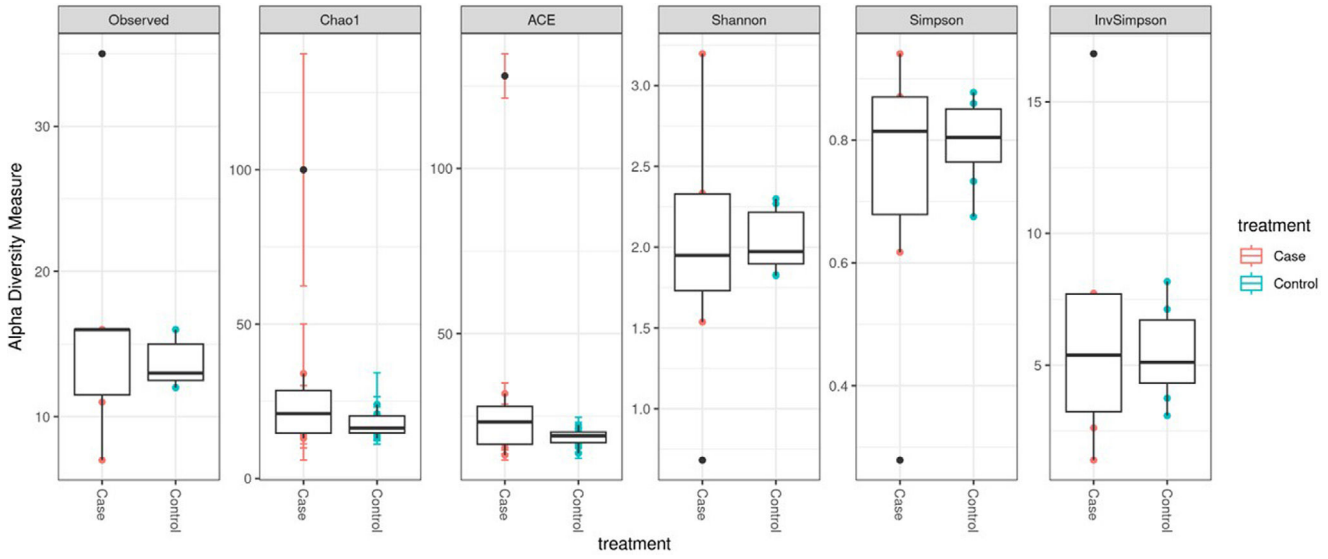

Figure 2 Comparison of average alpha-diversity scores in our study between cases and controls. Box plots show the average alpha-diversity scores within the two groups, which were measured by using Observed_OTUs, Chao 1, ACE, Shannon, Simpson, and InvSimpson.

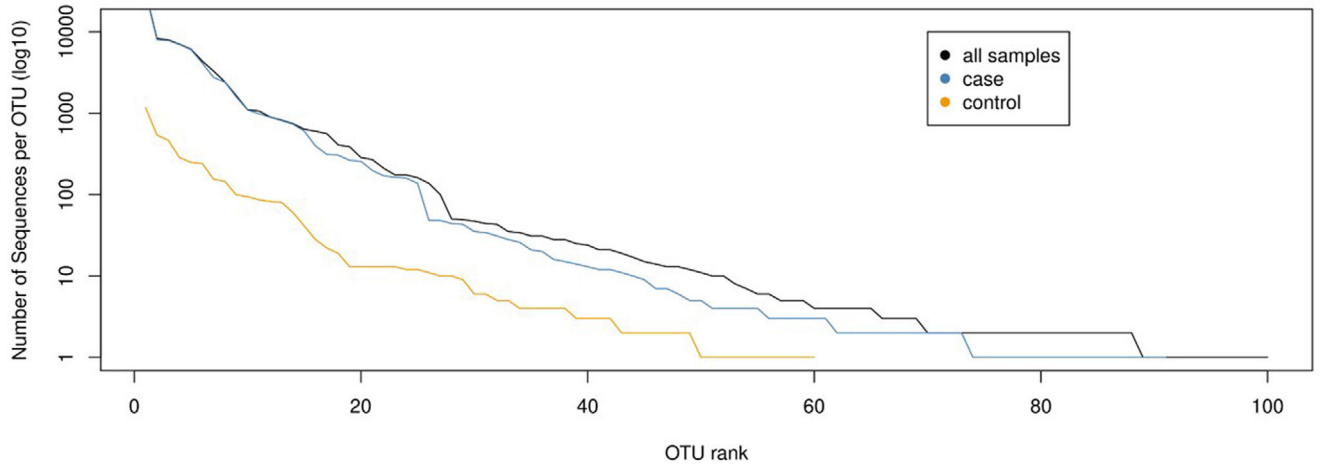

Figure 3 The abundances of the top 100 OTUs for the cases and controls communities in our study were ranked by abundance for each group.

gut microbiota is primarily composed of Bacteroidetes and Firmicutes[46]. The present study reported a low abundance of *Bacilli* (phylum Firmicutes), Lactobacillaceae, particularly genus *Lactobacillus* as well as a higher abundance of *Gammaproteobacteria*, Enterobacteriaceae, especially *Escherichia* in SLE samples compared to the controls. The beta-diversity investigation based on the Bray–Curtis distance revealed that the two groups share their abundant taxa but not the rare ones, which are different.

Bacillus and *Lactobacillus* strains are enteric bacteria and are widely used as probiotics[11]. *Bacillus* strains promote the growth of *Lactobacillus* to balance the microbiota and achieve therapeutic purposes[32].

Proteobacteria are Gram-negative bacteria that include many pathogens such as *Escherichia coli*, *Salmonella*, *Vibrio*, *Helicobacter*, and *Shigella*. The gut microbiota dominated by the genera *Escherichia/Shigella* is associated with low short-chain fatty acid concentrations and an increase in metabolic pathways, creating a pathogenic and inflammatory environment in these patients[6]. The lipopolysaccharides of Gammaproteobacteria are more immunogenic compared to those of some commensal Gram-negative bacteria, such as Bacteroides species. Enterobacteriaceae has been previously linked to intestinal inflammation[17], reflecting the common inflammatory response observed in SLE patients.

Similar results were published by He et al. (2020) in a study of 21 Chinese women with SLE and 10 healthy volunteers. They reported an increase in *Proteobacteria* (class Gammaproteobacteria, order Enterobacteriales, and family Enterobacteriaceae) in SLE patients and *Escherichia/Shigella*[14].

Hevia et al. studied 20 Spanish women with SLE of Caucasian origin and 20 healthy controls. They found a

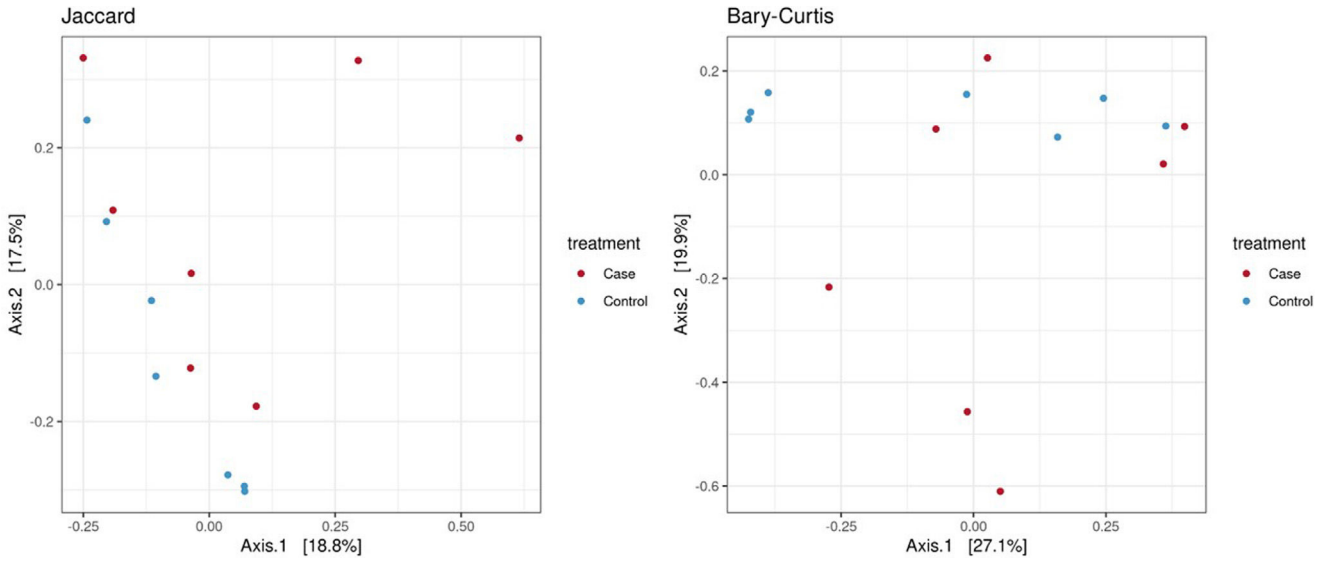

Figure 4 Beta-diversity analysis between the two groups (cases and controls) in our study based on Jaccard and Bray–Curtis distance calculation.

significantly lower Firmicutes/Bacteroidetes ratio in SLE patients (median ratio, 1.97) compared to healthy controls. The normalized abundances of classes *Bacteroidia* (phylum Bacteroidetes), *Clostridia* (phylum Firmicutes), as well as orders Bacteroidales and Clostridiales, are significantly different between the SLE patients and healthy controls. Lachnospiraceae and Ruminococcaceae (family level) were positively associated with the healthy controls[16].

He et al. (2016) showed that 45 Chinese female lupus patients presented an intestinal microbiota profile indicative of dysbiosis compared to 48 volunteers. The Firmicutes:Bacteroidetes ratio was decreased in patients compared to the healthy controls. Indeed, an abundance in the number of bacteria of the genera *Rhodococcus*, *Eubacterium*, *Flavonifractor*, *Eggerthella*, *Klebsiella* and *Prevotella* was significantly high in lupus patients, whereas the bacteria of the genera *Pseudobutyrivibrio* and *Dialister* exhibited low abundance. The intestinal microbiota (IM) in SLE patients includes a high abundance of Gram-negative bacteria and other types of bacteria, such as those of the genera *Blautia*, *Odoribacter*, and a genus from the family *Rikenellaceae*, with lower diversity than in controls[15].

Li et al. (2019) investigated the intestinal microbiota in 40 Chinese female SLE patients and 22 healthy controls. They reported a lower Firmicutes/Bacteroidetes (F/B) ratio in the feces of remissive SLE patients compared to healthy controls. Among the altered microbiota, the genera *Campylobacter*, *Streptococcus*, *Veillonella*, and the species *S. dispar* and *S. anginosus* were positively correlated with lupus activity, whereas the genus *Bifidobacterium* was negatively associated with disease activity[24].

In another study, van der Meulen et al. examined the intestinal microbiota of 30 white SLE patients from the Netherlands with a European ethnic background and 965 controls from the general population. They also found a lower Firmicutes/Bacteroidetes ratio in SLE patients compared to controls. The relative abundance of the phylum Bacteroidetes and the genus *Bacteroides* was significantly higher in SLE patients compared to the general population controls. Additionally, the genus *Alistipes* (belonging to Bacteroidetes) and the phylum Proteobacteria showed a notably higher relative abundance in SLE patients compared to controls[49].

Luo et al. studied the composition of IM in 14 African American (not Caribbean) and Caucasian, non-Hispanic lupus patients and 17 healthy controls and showed a high abundance of the phylum Proteobacteria in patients. Other species were more abundant in lupus patients, mainly *Odoribacter* and *Blautia* (family Lachnospiraceae). The Firmicutes/Bacteroidetes ratios were not significantly different for SLE patients in remission versus non-SLE controls[28].

Bacteroidetes and Firmicutes are the two most abundant bacterial phyla in the human gut. Hevia et al. reported a decrease in the Firmicutes/Bacteroidetes (F/B) ratio in SLE patients[16]. In contrast, other research groups found no alteration in the F/B ratio of SLE patients[15,28]. Our results, however, revealed a 1.29-fold increase in the F/B ratio in SLE patients (1.29 in cases compared to 0.81 in controls), along with a non-significant decrease in the abundance of Bacteroidetes.

Intestinal dysbiosis is also characterized by an increase in the F/B ratio in obesity[2]. Conversely, a lower F/B ratio is evident in other autoimmune diseases[31,35]. The F/B ratio decreases in patients with type 2 diabetes compared to controls[21]. Additionally, most studies on patients with Crohn's disease report a decrease in the abundance of Firmicutes and an increase in Bacteroidetes[21]. This imbalance in the F/B ratio appears to be a key feature of SLE dysbiosis, regardless of lifestyle, disease duration or stage, or diet.

Among the altered/disordered microbiota in our study, the genus *Clostridium_XVIII* (Firmicutes phylum) was negatively correlated with SLEDAI scores, while the class Betaproteobacteria (Proteobacteria phylum) and the genus *Faecalibacterium* (Firmicutes phylum) were positively associated with renal manifestations. The genus *Odoribacter*

(Bacteroidetes phylum) was positively correlated with disease duration, while the genus *Prevotella* (Bacteroidetes phylum) was negatively correlated with BMI index. Phyla Firmicutes and Bacteroidetes were involved in host metabolism and immunity according to Round et al.[39].

Faecalibacterium is a key member of the core functional group of the gut microbiota, playing several crucial roles in maintaining human health. These roles include generating energy components and nourishing the intestinal epithelium, mitigating inflammation severity, preserving intestinal barrier functions, and enhancing colon motility[10]. Recent findings suggest that *Faecalibacterium* can also alleviate renal dysfunction in chronic kidney disease patients through butyrate-mediated GPR43 signaling in the kidneys[23]. Elevated levels in SLE patients might be a compensatory response to improve renal symptoms.

Clostridium sp. can metabolize daidzein, an isoflavone shown to reduce inflammation in lupus-prone mice, potentially influencing SLE by decreasing daidzein levels[8]. Additionally, it catabolizes tryptophan, consumed by systemically activated T cells, via the kynurenine pathway, which has been found to be altered in SLE patients[1,33].

In our study, *Bifidobacterium* is less abundant in SLE patients with active disease compared to healthy controls. Liu et al. showed that short-chain fatty acids generated by *Bifidobacterium* and *Lactobacillus* combine and activate receptors (FFAR2, FFAR3, or GPR109a) on enterocytes to inhibit inflammatory responses by blocking nuclear factor-κ-light chain enhancer of B cells activation pathway. This point may explain the inflammatory response in SLE patients[25].

In our study, we observed some similarities with previous studies in bacterial microbiota but also noted differences likely due to variations in inclusion criteria. We included only female patients with a SLEDAI score <22 and also investigated the bacterial gut in SLE patients with active disease (SLEDAI >5), while Luo et al. and van der Meulen et al. included both male and female SLE patients of different ethnicities and with various levels of disease severity. Hevia et al.[16] included only Caucasian female patients with SLEDAI scores <8 considering the remission course. Moreover, we included SLE patients requiring immunosuppressive, immunomodulatory therapies, or who had discontinued treatment, whereas Hevia et al.[16] excluded those receiving immunological or steroid treatments. Our exclusion/inclusion criteria likely selected subjects who better represent the SLE patient population. This highlights the importance of considering the country of origin in microbiota comparisons and exercising caution in cross-cohort analyses from different countries due to strong geographical influences, mainly attributed to associated eating habits and dietary diversity, which significantly influence bacterial composition.

In our study, five of seven patients were treated with hydroxychloroquine and six of seven patients were treated with steroids. Angelakis et al.[4] and Pan et al.[37] showed that short-term/high-dose or long-term use of hydroxychloroquine can also alter the gut microbiota, resulting in a decreased level in the relative abundance of Firmicutes. We did not find a decreased in relative abundance of Firmicutes in SLE patients treated with hydroxychloroquine compared to those not receiving this treatment. However, some commonly used medicines to treat patients with SLE, such as steroids and methotrexate, can inhibit the growth of certain gut bacteria or have been previously associated with reduced diversity of the gut microbiota[30].

Wu et al. (2011) reported that dietary interventions, including whole grains, increase the Firmicutes/Bacteroidetes ratio and that Firmicutes levels were positively associated with a low-fat/high-fiber diet. In our study, there were no significant differences in age, dietary intake, and lifestyle factors (smoking, alcohol consumption, physical activity, and use of vitamin and mineral supplements [data not shown]) between SLE patients and healthy controls, reducing the likelihood that these factors influenced our gut microbial profile analysis[50].

SLE is characterized by abnormal antibody responses to nuclear and cytoplasmic antigens, including mainly anti-nuclear antibodies and anti-double-stranded DNA. Sample classification revealed no specific association between bacterial clustering and different clinical features or antibodies. The development of SLE is due to a dysbiotic gut microbiota, leading to compromised gut mucosal barrier control and facilitating antigen transit. Additionally, the production of autoantibodies can result from molecular mimicry with different bacterial populations in SLE. Bacteria have been found mimicking orthologous epitopes similar to host proteins, thereby activating autoimmune T and B cells[41].

We identified a specific type of microbiota for the SLE group. There are a few limitations to this study, most notably the small sample size, which was influenced by challenges in sample collection and exclusion criteria. Therefore, this may be insufficient to generate reproducible statistics. However, the strengths of our study lie in the investigation of the gut microbiota in female SLE patients with active disease receiving immunosuppressive treatment. Furthermore, our population is considered homogenous. Increasing the number of SLE patients and investigating the species level and the crosstalk between commensal bacteria, metabolites and the immune system may be crucial for understanding the immunopathology of this disease.

CRediT authorship contribution statement

Amira Messadi: writing, statistical analysis
Sameh Sayhi: revision, clinical evaluation
Kais Ghedira: bioinformatic analysis
Chadia Zaouaoui: data analysis
Bilel Arfaoui: clinical evaluation
Soumaya Khouikhi: revision
Maha Rebai: clinical evaluation
Nour El Houda Guediche: clinical evaluation
Nadia Abdelhafidh: revision
Bassem Louzir: clinical evaluation
Ajili Faida: revision and validation

Ethical considerations

The protocol of this study was approved by the Ethics Committee of the Military Hospital of Tunis.

Funding

This study was funded by department resources.

Conflict of interest

There is no conflict of interest.

Acknowledgments

The authors would like to thank Dr Imen Nahdi, the CEO of African Biotechnology Society and her collaborators for making available their laboratory and required equipment to perform the study and for performing a part of bioinformatic analysis. This study was funded by department resources.

References

1. Akesson K, Pettersson S, Stahl S, Surowiec I, Hedenström M, Eketjäll S, Trygg J, Jakobsson P, Gunnarsson I, Svenungsson E, Idborg H. Kynurenine pathway is altered in patients with SLE and associated with severe fatigue. Lupus Sci Med. 2018;5:e000254, http://dx.doi.org/10.1136/lupus-2017-000254.
2. Al-Assal K, Cristina Martinez CA, Torrinhas RS, Cardinelli C, Waitzberg D. Gut microbiota and obesity. Clin Nutr Exp. 2018;20:60–4, http://dx.doi.org/10.1016/j.yclnex.2018.03.001.
3. Alkanani AK, Hara N, Gottlieb PA, Ir D, Robertson CE, Wagner BD, Frank DN, Zipris D. Alterations in intestinal microbiota correlate with susceptibility to type 1 diabetes. Diabetes. 2015;64:3510–20, http://dx.doi.org/10.2337/db14-1847.
4. Angelakis E, Million M, Kankoe S, Lagier JC, Armougom F, Giorgi R, Raoult D. Abnormal weight gain and gut microbiota modifications are side effects of long-term doxycycline and hydroxychloroquine treatment. Antimicrob Agents Chemother. 2014;58:3342–7.
5. Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrøm K, Massarotti E, McCune J, Ruiz-Irastorza G, Sanchez-Guerrero J, Schneider M, Urowitz M, Bertsias G, Hoyer BF, Leuchten N, Tani C, Tedeschi SK, Touma Z, Schmajuk G, Anic B, Assan F, Chan TM, Clarke AE, Crow MK, Czirják L, Doria A, Graninger W, Halda-Kiss B, Hasni S, Izmirly PM, Jung M, Kumánovics G, Mariette X, Padjen I, Pego-Reigosa JM, Romero-Diaz J, Rúa-Figueroa Fernández Í, Seror R, Stummvoll GH, Tanaka Y, Tektonidou MG, Vasconcelos C, Vital EM, Wallace DJ, Yavuz S, Meroni PL, Fritzler MJ, Naden R, Dörner T, Johnson SR. European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019;71:1400–12, http://dx.doi.org/10.1002/art.40930.
6. Baltazar-Díaz TA, González-Hernández LA, Aldana-Ledesma JM, Peña-Rodríguez M, Vega-Magaña AN, Zepeda-Morales ASM, López-Roa RI, Del Toro-Arreola S, Bueno-Topete MR. Notable intestinal dysbiosis orchestrated by *Escherichia/Shigella*, decreased levels of SCFTA (short chain fatty acids) and alterations in metabolic pathways characterize patients with alcohol-decompensated cirrhosis in western Mexico. Ann Hepatol. 2023;27(S3), http://dx.doi.org/10.1016/j.aohep.2022.100812.
7. Chappidi S, Villa EC, Cantarel BL. Using Mothur to determine bacterial community composition and structure in 16S ribosomal RNA datasets. Curr Protoc Bioinformatics. 2019;67:e83, http://dx.doi.org/10.1002/cpbi.83.
8. Chen B, Jia X, Xu J, Zhao LD, Ji JY, Wu BX, Ma Y, Li H, Zuo XX, Pan WY, Wang XH, Ye S, Tsokos GCT, Wang J, Zhang X. An autoimmunogenic and proinflammatory profile defined by the gut microbiota of patients with untreated systemic lupus erythematosus. Arthritis Rheumatol. 2021;73:232–43, http://dx.doi.org/10.1002/art.41511.
9. 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. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. Sci Rep. 2016;6:28484, http://dx.doi.org/10.1038/srep28484.
10. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res. 2013;54:2325–40, http://dx.doi.org/10.1194/jlr.R036012.
11. Elshaghabee FM, Rokana N, Gulhane RD, Sharma C, Panwar H. *Bacillus* as potential probiotics: status, concerns, and future perspectives. Front Microbiol. 2017;8:1490, http://dx.doi.org/10.3389/fmicb.2017.01490.
12. Fassatoui M, Lopez-Siles M, Dıaz-Rizzolo DA, Jmel H, Naouali C, Abdessalem G, Chikhaoui A, Nadal B, Jamoussi H, Abid A, Gomis R, Abdelhak S, Martinez-Medina M, Kefi R. Gut microbiota imbalances in Tunisian participants with type 1 and type 2 diabetes mellitus. Biosci Rep. 2019;39, http://dx.doi.org/10.1042/BSR20182348. BSR20182348.
13. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JG, Relman DA, Fraser-Liggett CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science. 2006;312:1355–9, http://dx.doi.org/10.1126/science.1124234.
14. He J, Chan T, Hong X, Zheng F, Zhu C, Yin L, Dai W, Tang D, Liu D, Dai Y. Microbiome and metabolome analyses reveal the disruption of lipid metabolism in systemic lupus erythematosus. Front Immunol. 2020;11:1703, http://dx.doi.org/10.3389/fimmu.2020.01703.
15. He Z, Shao T, Li H, Xie Z, Wen C. Alterations of the gut microbiome in Chinese patients with systemic lupus erythematosus. Gut Pathog. 2016;8:64, http://dx.doi.org/10.1186/s13099-016-0146-9.
16. Hevia A, Milani C, López P, Cuervo A, Arboleya S, Duranti S, Turroni F, González S, Suárez A, Gueimonde M, Ventura M, Sánchez B, Margolles A. Intestinal dysbiosis associated with systemic lupus erythematosus. MBio. 2014;5, http://dx.doi.org/10.1128/mbio.01548-14, e01548-14.
17. Hughes ER, Winter MG, Duerkop BA, Luisella Spiga L, Tatiane Furtado de Carvalho TF, Zhu W, Gillis CC, Büttner L, Smoot MP, Behrendt CL, Cherry S, Santos RL, Hooper LV, Winter SE. Microbial respiration and formate oxidation as metabolic signatures of inflammation-associated dysbiosis. Cell Host Microbe. 2017;21:208–19, http://dx.doi.org/10.1016/j.chom.2017.01.005.
18. Jangi S, Gandhi R, Cox L, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Shirong Liu S, Glanz BL, Cook S, Tankou S, Stuart F, Melo K, Nejad P, Smith K, Topçuolu B, Holden J, Kivisäkk P, Chitnis T, Jager PL, Quintana FJ, Gerber G, Bry L, Weiner HL. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun. 2016;7:12015, http://dx.doi.org/10.1038/ncomms12015.
19. Kharfi M, Elloumi L, Khorchani H, Goucha S, Fazaa B, Kamoun MR. Lupus eythémateux chronique. Etude épidémio_clinique. Maghreb Médical. 1997;316:29–30.
20. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology. 2014;146:1489–99, http://dx.doi.org/10.1053/j.gastro.2014.02.009.
21. Larsen N, Vogensen FK, van den Beg FW, Nielsen DS, Andreasen AS, Pedersen BK, Abu Al-Soud W, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 dia-

betes differs from nondiabetic adults. PLoS One. 2010;5:e9085, http://dx.doi.org/10.1371/journal.pone.0009085.
22. Letaief H, El Euch D, Mokni M, Trojjet S, Ben Osman A. Lupus eythémateux chronique: à propos de 206 cas. Nouv Dermatol. 1999;18:34–6.
23. Li HB, Xu ML, Xu XD, Tang YY, Jiang HL, Li L, Xia WJ, Cui N, Bai J, Dai ZM, Han B, Li Y, Peng B, Dong YY, Aryal S, Manandhar I, Eladawi MA, Shukla R, Kang YM, Joe B, Yang T. *Faecalibacterium prausnitzii* attenuates CKD via butyrate-renal GPR43 axis. Circ Res. 2022;131:e120–34, http://dx.doi.org/10.1161/CIRCRESAHA.122.
24. Li Y, Wang HF, Li X, Li HX, Zhang Q, Zhou H, He Y, Li P, Fu C, Zhang X, Qiu YR, Li JL. Disordered intestinal microbes are associated with the activity of systemic lupus erythematosus. Clin Sci (Lond). 2019;133:821–38, http://dx.doi.org/10.1042/CS20180841.
25. Liu Y, Alookaran JJ, Rhoads JM. Probiotics in autoimmune and inflammatory disorders. Nutrients. 2018;10:1537, http://dx.doi.org/10.3390/nu10101537.
26. Llorente C, Schnabl B. The gut microbiota and liver disease. Cell Mol Gastroenterol Hepatol. 2015;1:275–84, http://dx.doi.org/10.1016/j.jcmgh.2015.04.003.
27. Lopez P, De Paz B, Rodriguez-Carrio J, Hevia A, Sánchez B, Margolles A, Suárez A. Th17 responses and natural IgM antibodies are related to gut microbiota composition in systemic lupus erythematosus patients. Sci Rep. 2016;6:24072, http://dx.doi.org/10.1038/srep24072.
28. Luo XM, Edwards MR, Mu Q, Yu Y, Vieson MD, Reilly CM, Ansar Ahmed S, Bankole SAAA. Gut microbiota in human systemic lupus erythematosus and a mouse model of lupus. Appl Environ Microbiol. 2018;84, http://dx.doi.org/10.1128/AEM.02288-17, e02288-17.
29. Mahfoudh A, Khaled A, Chtourou O, Kharfi M, Zeglaoui F, Fazaa B, Kamoun MR. Le lupus erythémateux chronique: 104 cas tunisiens. La Tunisie Medicale. 2010;88:742–5 [PMID: 20890823].
30. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Hitomi Dose H, Mori H, Patil KR, Bork P, Typas A. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018;555:623–8.
31. Man SM, Kaakoush NO, Mitchell HM. The role of bacteria and pattern-recognition receptors in Crohn's disease. Nat Rev Gastroenterol Hepatol. 2011;8:152–68, http://dx.doi.org/10.1038/nrgastro.2011.3.
32. Mingmongkolchai S, Panbangred W. *Bacillus* probiotics: an alternative to antibiotics for livestock production. J Appl Microbiol. 2018;124:1334–46, http://dx.doi.org/10.1111/jam.13690.
33. Miyajima M, Zhang B, Sugiura Y, Sonomura K, Guerrini MM, Tsutsui Y, Maruya M, Vogelzang A, Chamoto K, Honda K, Hikida T, Ito S, Qin H, Sanuki R, Suzuki K, Furukawa T, Ishihama Y, Matsuda F, Suematsu M, Honjo T, Fagarasan S. Metabolic shift induced by systemic activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional behavior. Nat Immunol. 2017;18:1342–52, http://dx.doi.org/10.1038/ni.3867.
34. Miyake S, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim S, Morita H, Hattori M, Yamamura T. Dysbiosis in the gut microbiota of patients with multiple sclerosis, with a striking depletion of species belonging to *clostridia* XIVa and IV clusters. PLoS One. 2015;10:e0137429, http://dx.doi.org/10.1371/journal.pone.0137429.
35. Murri M, Leiva I, Gomez-Zumaquero JM, Tinahones FJ, Cardona F, Soriguer F, Queipo-Ortuño MI. Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case–control study. BMC Med. 2013;11:46, http://dx.doi.org/10.1186/1741-7015-11-46.
36. Pan Q, Guo F, Huang Y, Li A, Chen S, Chen J, Liu HF, Pan Q. Gut microbiota dysbiosis in systemic lupus erythematosus: novel insights into mechanisms and promising therapeutic strategies. Front Immunol. 2021;12:799788, http://dx.doi.org/10.3389/fimmu.2021.799788.
37. Pan Z-Y, Chang Y-X, Han N, Hou FY, Lee BY, Zhi FC, Yang RF, Bi YJ. Short-term high-dose gavage of hydroxychloroquine changes gut microbiota but not the intestinal integrity and immunological responses in mice. Life Sci. 2021;264:118450.
38. Quast C, Pruesse E, Yilmaz P, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590–6, http://dx.doi.org/10.1093/nar/gks1219.
39. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol. 2009;9:313–23, http://dx.doi.org/10.1038/nri2515.
40. Ruiz L, López P, Suárez A, Sánchez B, Margolles A. The role of gut microbiota in lupus: what we know in 2018? Expert Rev Clin Immunol. 2018;14:787–92, http://dx.doi.org/10.1080/1744666X.2018.1519395.
41. Sai A, Shetty GB, Shetty P, Nanjeshgowda HL. Influence of gut microbiota on autoimmunity: a narrative review. Brain Behav Immun Integrative. 2024;5:100046, http://dx.doi.org/10.1016/j.bbii.2024.100046.
42. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR. Expansion of intestinal *Prevotella copri* correlates with enhanced susceptibility to arthritis. Elife. 2013;2:e01202, http://dx.doi.org/10.7554/eLife.01202.
43. Selber-Hnatiw S, Rukundo B, Ahmadi M, Akoubi H, Al-Bizri H, Aliu AF, Ambeaghen TU, Avetisyan L, Bahar I, Baird A, Begum F, Ben Soussan H, Blondeau-Éthier V, Bordaries R, Bramwell H, Briggs A, Bui R, Carnevale M, Chancharoen M, Chevassus T, Choi JH, Coulombe K, Couvrette F, D'Abreau S, Davies M, Desbiens MP, Di Maulo T, Di Paolo S, Do Ponte S, dos Santos Ribeiro P, Dubuc-Kanary L, Duncan PK, Dupuis F, El-Nounou S, Eyangos C, Ferguson NK, Flores-Chinchilla NR, Fotakis T, Oumarou HD, Georgiev MG, Ghiassy M, Glibetic S, Julien Grégoire Bouchard N, Hassan JG, Huseen T, Quilatan I, Iozzo MI, Islam T, Jaunky S, Jeyasegaram DB, Johnston A, Kahler M, Kaler MR, Kamani K, Rad C, Konidis HK, Konieczny E, Kurianowicz F, Lamothe S, Legros P, Leroux K, Li S, Rodriguez J, Luponio-Yoffe MER, Yara Maalouf S, Jessica Mantha Y, Melissa McCormick J, Mondragon M, Narayana P, Neretin T, Nguyen E, Niu TTT, Nkemazem I, O'Donovan RB, Oueis M, Paquette M, Patel S, Pecsi N, Peters E, Pettorelli J, Poirier A, Pompa C, Rajen VR, Ralph H, Rosales-Vasquez RO, Rubinshtein J, Surya Sakr D, Sebai S, Serravalle MS, Sidibe L, Sinnathurai F, Soho A, Sundarakrishnan D, Svistkova A, Ugbeye V, Vasconcelos TE, Vincelli MS, Voitovich M, Vrabel O, Wang P, Wasfi L, Zha M, Gamberi CY. Human gut microbiota: toward an ecology of disease. Front Microbiol. 2017;8:1265, http://dx.doi.org/10.3389/fmicb.2017.01265.
44. Siegel CH, Sammaritano LR. Systemic lupus erythematosus: a review. JAMA. 2024;331:1480–91, http://dx.doi.org/10.1001/jama.2024.2315.
45. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. Inflamm Bowel Dis. 2009;15:1183–9, http://dx.doi.org/10.1002/ibd.20903.
46. Toumi E, Goutorbe B, Plauzolles A, Bonnet M, Mezouar S, Militello M, Jean-Louis Mege JL, Chiche L, Halfon P. Gut microbiota in systemic lupus erythematosus patients and lupus mouse model: a cross species comparative analysis for biomarker discovery. Front Immunol. 2022;13:943241, http://dx.doi.org/10.3389/fimmu.2022.943241.
47. Toumi E, Mezouar S, Plauzolles A, Chiche L, Bardin N, Halfon P, Mege JL. Gut microbiota in SLE: from animal models to clinical evidence and pharmaco-

logical perspectives. Lupus Sci Med. 2023;10:e000776, http://dx.doi.org/10.1136/lupus-2022-000776.
48. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011;365:2110–21, http://dx.doi.org/10.1056/NEJMra1100359.
49. van der Meulen TA, Harmsen HJM, Vila AV, Kurilshikov A, Liefers SC, Zhernakova A, Fu J, Wijmenga C, Weersma RK, de Leeuw K, Bootsma H, Spijkervet FKL, Vissink A, Kroese FGM. Shared gut, but distinct oral microbiota composition in primary Sjögren's syndrome and systemic lupus erythematosus. J Autoimmun. 2019;97:77–87, http://dx.doi.org/10.1016/j.jaut.2018.10.009.
50. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334:105–8, http://dx.doi.org/10.1126/science.1208344.
51. Zhang L, Qing P, Yang H, Wu Y, Liu Y, Luo Y. Gut microbiome and metabolites in systemic lupus erythematosus: link, mechanisms and intervention. Front Immunol. 2021;12:686501, http://dx.doi.org/10.3389/fimmu.2021.686501.