Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2025**; 52(11): 45531
https://doi.org/10.31083/CEOG45531

Original Research

Deciphering the Pain-Related Gut Microbiome in Patients With Endometriosis

Wen Shi[1,2,3,†], Minyi Wang[1,2,3,†], Zhuang Jin[1,2,3], Huiling Lai[1,2,3], Xiaochuan Chen[1,2,3], Xiao Li[1,2,3], Qiyu Zhong[1,2,3], Jianyu Ma[1,2,3], Lingbing Qiu[1,2,3], Shuqin Chen[1,2,3,*]

[1]Department of Gynecology, The Sixth Affiliated Hospital, Sun Yat-sen University, 510655 Guangzhou, Guangdong, China
[2]Key Laboratory of Human Microbiome and Chronic Diseases (Sun Yat-sen University), Ministry of Education, 510655 Guangzhou, Guangdong, China
[3]Biomedical Innovation Center, The Sixth Affiliated Hospital, Sun Yat-sen University, 510655 Guangzhou, Guangdong, China
*Correspondence: chshqin@mail.sysu.edu.cn (Shuqin Chen)
[†]These authors contributed equally.
Academic Editor: Andrea Tinelli
Submitted: 4 August 2025 Revised: 7 September 2025 Accepted: 19 September 2025 Published: 31 October 2025

Abstract

Background: Endometriosis (EMS), which affects >10% of women, is characterized by painful symptoms, such as dysmenorrhea. Meanwhile, the gut microbiota is linked to EMS; however, the relationship between the gut microbiota and EMS-related pain remains unclear. **Methods**: This study conducted 16S rRNA sequencing on fecal samples from 20 patients with EMS and dysmenorrhea, 13 patients with EMS but not dysmenorrhea, and 12 healthy controls. **Results**: Carbohydrate antigen 125 (CA125) levels were significantly higher in patients with EMS and dysmenorrhea. No significant differences in α- and β-diversity values were observed among the three groups ($p > 0.05$). However, *Proteobacteria* and *Subdoligranulum* showed increased abundance trends in patients with EMS and dysmenorrhea. *Acinetobacter* and *Colidextribacter* were less abundant in patients with EMS and dysmenorrhea than in those without dysmenorrhea ($p < 0.05$). *Faecalicoccus pleomorphus* and its genus also showed consistent depletion. At the genus level, a correlation analysis of the differential microbes revealed that *UCG_005* and *UCG_002* were central nodes in the correlation network ($p < 0.05$). Functional prediction indicated significant enrichment in cofactor and vitamin metabolism pathways in the EMS group with dysmenorrhea ($p < 0.05$). Several differential microbes, such as *Faecalicoccus*, *Colidextribacter*, and *Acinetobacter*, were negatively correlated with pain severity. Receiver operating characteristic curve analysis identified 10 microbial species with moderate diagnostic potential (area under the curve >0.7) for discriminating patients with EMS and dysmenorrhea, notably *Ligilactobacillus*. **Conclusions**: These results highlight distinct microbial alterations and functional pathways associated with EMS-related pain, suggesting potential diagnostic biomarkers for this condition.

Keywords: endometriosis; gut microbiome; endometriosis-related pain

1. Introduction

Endometriosis (EMS) is a chronic estrogen-dependent disorder affecting >10% of women, characterized by ectopic endometrial-like tissue growth and pelvic inflammation [1]. This condition often leads to infertility, chronic pelvic pain, and dysmenorrhea [2]. Dysmenorrhea is defined as cyclic lower abdominal cramping radiating to the thighs or lower back with accompanying nausea, diarrhea, and malaise [3] and demonstrates bimodal classification. The discomfort typically sets in before or at the onset of menstruation, persisting for 8–72 h [4]. The condition is classified into primary and secondary dysmenorrhea. Primary dysmenorrhea lacks identifiable pelvic pathology, whereas secondary forms stem from underlying conditions–predominantly EMS [4]. Pathophysiologically, dysmenorrhea is driven by vascular contraction and inflammatory responses, and prostaglandins, leukotrienes, and vasopressin were identified as key mediators [5]. Regarding management, nonsteroidal anti-inflammatory drugs and oral contraceptives are widely recognized as first-line treatments to alleviate symptoms of dysmenorrhea. They reduce inflammation and mitigate the effects of prostaglandins, respectively. However, these interventions have limited efficacy in dysmenorrhea associated with organic lesions such as EMS. Other therapeutic options also have notable limitations [6]. Given that women with secondary dysmenorrhea often experience persistent and worsening pain over time [4], elucidating the underlying regulatory mechanisms of dysmenorrhea is crucial for developing more effective treatments.

The gut microbiota has been implicated in EMS, and emerging evidence positions EMS within the gut-microbiota-disease axis, which demonstrated microbial dysbiosis in EMS patients [7,8]. Mechanistic studies suggest that the microbiota influences EMS progression through estrogen metabolism, immune modulation, and cytokine regulation [9]. Gut-derived lipopolysaccharides may activate macrophage-mediated inflammatory

cascades, promoting EMS lesion angiogenesis and proliferation [10]. In addition, gut microbiota was found to be related to pain. Notably, the gut microbiota modulates pain perception via central and peripheral pathways, balancing pro-/anti-inflammatory mediators and visceral hypersensitivity [11]. Moreover, a study has suggested that probiotic intake may alleviate stress-induced visceral hypersensitivity [12]. In particular, butyrate produced by *Roseburia intestinalis* was found to attenuate neuropathic pain [13]. In mouse models of primary dysmenorrhea, the gut microbiota is significantly altered. Specifically, the abundance of *Lactobacillus* is reduced coupled with an increase in *Romboutsia* [14]. However, relatively few studies have examined the gut microbiota in EMS-related secondary dysmenorrhea.

This study aimed to identify microbial markers associated with EMS-related dysmenorrhea. A comparative analysis of the gut microbiomes of women with EMS was conducted, distinguishing between those who experienced dysmenorrhea and those who did not and comparing them with a group of healthy controls to discover potential avenues for reducing pain symptoms in EMS through gut microbiome regulation.

2. Methods

2.1 Study Participants

Fecal specimens were prospectively collected from June to December 2023 at The Sixth Affiliated Hospital of Sun Yat-Sen University, comprising three patient cohorts: (1) EMS with dysmenorrhea (n = 20; median age, 31.5 years), (2) EMS without dysmenorrhea (n = 13; median age, 28.0 years), and (3) healthy controls (n = 12; median age, 28.5 years).

Ethical approval was obtained from the institutional review board (2024ZSLYEC-020), and written informed consent was obtained from all participants. Pain severity was assessed using the 11-point numerical rating scale, where participants rated their average pain during the past three menstrual cycles on a 0–10 scale.

The specific inclusion and exclusion criteria for the study were as follows:

Inclusion criteria for patients with EMS:

1. Aged 18–45 years, enrolled during the nonmenstrual, nonpregnant, and nonpuerperal periods, with regular menstrual cycles.

2. Diagnostically confirmed by ultrasonography or pelvic magnetic resonance imaging (MRI, both with sensitivity and specificity >90%) [15].

3. No hormone therapy in the past six months and did not take antibiotics or probiotics within the past eight weeks.

4. Non-smokers.

5. Absence of inflammatory or nerve-related pain conditions.

6. No history of intestinal surgery, no intestinal treatments within the past six months, and no significant family history of major intestinal diseases (e.g., colorectal cancer or inflammatory bowel disease).

7. Not in the acute stage of vaginitis, with a negative cervical human papillomavirus test.

8. No special dietary habits (e.g., vegan or vegetarian diet, preference for spicy foods, or highly acidic diet).

The diagnosis was based on characteristic findings on transvaginal ultrasonography (unilocular cyst without vascularity and homogeneous ground-glass echogenicity) and/or MRI (high T1 signal, T2 shading, T2 dark spots, and evidence of adhesions), which are widely recognized as reliable alternatives to laparoscopy for this EMS subtype.

Inclusion criteria for healthy controls:

Healthy controls were recruited with the same eligibility criteria as patients with EMS, except that they had regular menstrual cycles without a history of EMS or dysmenorrhea.

The exclusion criteria were as follows:

1. Have other chronic abdominal or kidney issues, ovarian/uterine tumors, liver diseases, inflammatory or immune conditions, or heart/coronary artery diseases.

2. Received hormone therapy within six months before surgery.

3. Regularly use painkillers.

4. Have a history of headaches or other neurological disorders.

5. Experienced severe pelvic inflammation during surgery.

6. Have severe chronic or acute inflammatory diseases.

7. Have adenomyosis.

2.2 16S rRNA Sequencing

DNA was extracted using a DNA extraction kit according to the manufacturer's protocol. The highly variable V3–V4 region of the bacterial 16S rRNA gene, identified by the primer set 338F–806R, was then targeted for amplification by polymerase chain reaction (PCR). The primer sequences employed were as follows: 338F 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R 5′-GGACTACHVGGTWTCTAAT-3′. The PCR-amplified products were visualized on agarose gels and then purified. The purified PCR amplicons were then pooled in equimolar concentrations for paired-end sequencing (2 × 250 bp) on the Illumina Novaseq 6000 platform (Illumina, San Diego, CA, USA).

2.3 Microbiome Data Processing and Analysis

Fecal specimens were initially collected on dry ice and subsequently stored at –80 °C until sequencing to ensure sample integrity. The raw sequencing data were initially processed based on an individual nucleotide quality assessment. The raw data were rigorously filtered using Trim-

momatic (version 0.33: http://www.usadellab.org/cms/?page=trimmomatic) [16]. Subsequently, primer sequences were identified and precisely excised with Cutadapt (version 1.9.1; https://cutadapt.readthedocs.io/en/stable/) [17]. The paired-end reads obtained from previous steps were assembled by USEARCH (version 10; https://www.drive5.com/usearch/) [18]. To ensure the reliability of the assembled sequences, chimeras were detected and removed by employing UCHIME (version 8.1; https://www.drive5.com/usearch/manual8.1/uchime_algo.html) [19].

By employing a 97% sequence similarity threshold, operational taxonomic units (OTUs) were derived from nonrepetitive sequences, except for single sequences, and clustered using USEARCH (version 10.0; http://www.drive5.com/usearch/). This stringent clustering approach resulted in a comprehensive OTU abundance table. OTUs were meticulously classified and annotated, leveraging the SILVA database (version 138.1; https://www.arb-silva.de/) [20] and employing the Naive Bayes classifier integrated within QIIME2 (v.2020.6; https://qiime2.org/) [21]. This classification was conducted with a confidence level set at 70%. To assess the α-diversity, the abundance-based coverage estimator (ACE), Chao1, and phylogenetic diversity (PD) whole tree indices were calculated. β-diversity, which quantifies the compositional dissimilarity between microbial communities, was explored using principal component analysis (PCA). To identify differential microorganisms across groups, Python scripts were employed to create ternary phase diagrams, the Wilcoxon rank-sum test was applied to test significant differential microorganisms between groups, and intersection analysis was applied to pinpoint co-regulated differential microbes. The functional potential of the microbial communities was deduced using PICRUSt (version v.2.5.2; https://picrust.github.io/picrust/), a bioinformatics tool that predicts metabolic pathways from 16S rRNA gene sequences. To further delve into the diversity of these metabolic pathways, the Cluster of Orthologous Groups database and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were referred to. Spearman correlation analysis was conducted to investigate the correlation between the gut microbiome and clinical indicators among patients with EMS-related pain. Ultimately, the predictive ability of the differentially abundant microorganisms was evaluated by the receiver operating characteristic (ROC) curve analysis. To identify the optimal threshold for discriminating between groups, sensitivity and specificity for each microbial marker were determined using the Youden index.

2.4 Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 10 (GraphPad Software, San Diego, CA, USA). The distribution of continuous variables was assessed using the Shapiro-Wilk test and visual inspection of quantile-quantile (Q-Q) plots (**Supplementary Fig. 1**). For normally distributed variables (age and body mass index [BMI]), the homogeneity of variances between groups was evaluated using the Brown-Forsythe test. Given that both variables satisfied the assumption of equal variances, they were compared between groups using the one-way analysis of variance. Non-normally distributed continuous variables with three groups (age at menarche [AAM], gravidity, parity, miscarriages, pain level, pain duration, anti-Müllerian hormone [AMH], carbohydrate antigen 125 [CA125], CA19-9, neutrophil count [N], lymphocyte count [L], and neutrophil-to-lymphocyte ratio [NLR]) were compared using the Kruskal-Wallis test, whereas volume of chocolate cysts (VOCC), which involves two groups, was compared using the Mann-Whitney U test. Categorical variables, such as the distribution of endometriotic cysts (unilateral vs. bilateral), were analyzed using Fisher's exact test. Continuous data are presented as mean $\pm$ standard deviation (SD) for normally distributed variables and as median [interquartile range, IQR] for non-normally distributed variables. A p-value < 0.05 was considered significant.

3. Results

3.1 Characteristics of the Study Participants

This study enrolled 45 women categorized into three groups: 20 patients with EMS and dysmenorrhea, 13 patients with EMS but without dysmenorrhea, and 12 healthy controls. Their baseline characteristics are shown in Table 1. No significant differences were observed among the three groups in terms of age, BMI, AAM, gravidity, parity, or miscarriage frequency (all $p > 0.05$). The distribution of endometriotic cysts (unilateral vs. bilateral) was not significantly different between the EMS group with and without dysmenorrhea ($p > 0.05$). All patients with EMS and dysmenorrhea experienced chronic pelvic pain, with a mean duration of 6.00 [3.25, 10.00] years and an average pain score of 5.00 [3.25, 7.00]. Serum CA125 levels were significantly higher in the EMS patients with dysmenorrhea compared with both the EMS group without dysmenorrhea and the healthy control group (Kruskal-Wallis statistic = 13.4; $p = 0.0012$). In contrast, no significant differences were found in AMH, CA19-9, N, L, or NLR among the groups (all $p > 0.05$).

3.2 Analysis of Gut Microbial Diversity and Structure

To explore potential relationships between dysmenorrhea and the gut microbiome in EMS, 16S rRNA sequencing was performed on 45 stool samples, identifying 5535 OTUs. Samples were categorized into three groups: control (healthy controls), EMS–Pain (EMS without dysmenorrhea), and EMS+Pain (EMS with dysmenorrhea). Rank-abundance and species accumulation curves indicated sufficient sequencing depth and adequate OTU coverage across the groups (Fig. 1A,B). The α-diversity analyses (ACE, Chao1, and PD whole tree) did not show significant pairwise differences among the groups (all $p > 0.05$; Fig. 1C–

Table 1. Characteristics of the study participants.

Characteristics	Control	EMS without dysmenorrhea	EMS with dysmenorrhea	*p* value
N	12	13	20	-
Age, years (Mean ± SD)	28.42 ± 4.17	29.38 ± 4.89	31.35 ± 5.22	0.2336 [a]
BMI, kg/m^2 (Mean ± SD)	20.53 ± 3.13	19.77 ± 1.92	19.45 ± 1.83	0.4329 [a]
AAM, years	14.00 [13.00, 14.75]	13.00 [13.00, 13.00]	13.00 [12.00, 14.00]	0.3932
Gravidity	0.00 [0.00, 0.75]	0.00 [0.00, 1.50]	0.00 [0.00, 1.75]	0.6384
Parity	0.00 [0.00, 0.00]	0.00 [0.00, 0.50]	0.00 [0.00, 1.00]	0.5099
Miscarriages	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.4951
Pain level	-	-	5.00 [3.25, 7.00]	-
Pain duration, years	-	-	6.00 [3.25, 10.00]	-
Unilateral/Bilateral endometriotic cysts (%)				0.4311 [b]
Unilateral	-	11 (84.6)	14 (70.0)	
Bilateral	-	2 (15.4)	6 (30.0)	
VOCC, mm^3	-	43,952.00 [2529.00, 128,658.00]	7683.00 [2457.00, 58,702.00]	0.2081 [c]
AMH, ng/mL	4.74 [3.54, 6.16]	3.95 [1.50, 5.65]	2.34 [1.88, 4.07]	0.0616
CA125, U/mL	15.70 [12.18, 24.35]	30.20 [23.35, 47.05]	48.80 [32.05, 100.80]	0.0012
CA19-9, U/mL	11.20 [4.93, 21.63]	11.03 [6.21, 18.50]	21.24 [11.37, 31.26]	0.1879
N, (×10^9/L)	3.28 [2.63, 4.52]	3.60 [3.01, 5.01]	3.54 [2.55, 4.27]	0.4407
L, (×10^9/L)	2.01 [1.79, 2.36]	1.82 [1.56, 2.47]	1.89 [1.34, 2.29]	0.3709
NLR	1.58 [1.15, 2.31]	1.97 [1.50, 2.50]	1.90 [1.45, 2.35]	0.3756

Data are represented as median [interquartile range]. N, number; SD, standard deviation; EMS, endometriosis; BMI, body mass index; AAM, age at menarche; VOCC, volume of chocolate cysts; AMH, anti-müllerian hormone; CA125, glycoconjugate antigen 125; CA19-9, glycoconjugate antigen 19-9; N, neutrophil; L, lymphocyte; NLR, neutrophil/lymphocyte ratio. Data were statistically analyzed using Kruskal-Wallis test, with the exception of the data labeled as [a], [b], and [c]. [a] indicates One-Way Analysis of Variance (ANOVA) tests, [b] indicates Fisher's exact test, and [c] indicates Mann-Whitney test.

E). PCA based on OTU composition showed no clear separation among the three groups; however, some distributional variations were observed (Fig. 1F). Principal component 1 (PC1) and principal component 2 (PC2) explained 13.35% and 12.52% of the variance, respectively. These findings suggest that potential group differences in gut microbiota may be subtle and are not reflected in overall community structure.

Then, composition analyses were conducted at both phylum and genus levels. At the phylum level, *Firmicutes* and *Bacteroidetes* were dominant across all groups. The relative abundance of *Proteobacteria* displayed a trend toward an increase in the EMS group with dysmenorrhea, whereas *Fusobacteriota* showed relatively lower abundance in the EMS group with dysmenorrhea compared with the other groups (Fig. 1G). At the genus level, *Bacteroides* and *Faecalibacterium* were relatively abundant (Fig. 1H). Specifically, the relative abundance levels of *Subdoligranulum* and *Parabacteroides* were higher in the EMS group with dysmenorrhea. Healthy controls exhibited higher *Roseburia* abundance than the EMS group (Fig. 1H). However, none of these microbial abundance variations reached significance. In summary, although overall diversity remained comparable, subtle group-specific shifts in the relative abundance of certain taxa may be associated with the dysmenorrhea status in EMS. Thus, further validation is necessary to confirm these potential associations.

3.3 Screening for Differential Microorganisms in EMS With Pain

To discern the gut microbes specifically implicated in EMS and EMS-related pain, differential species analysis was conducted using taxonomically annotated data from the five most abundant phyla and other phyla. The ternary diagram showed uniform abundance and distribution of *Bacteroidota* and *Firmicutes* across three cohorts (Fig. 2A). Wilcoxon rank-sum testing identified 12 genera with the top smallest *p*-values, with *Colidextribacter* exhibiting the highest relative abundance. Compared with the EMS group without dysmenorrhea, the abundances of *Colidextribacter* and *Acinetobacter* were significantly decreased in the EMS group with dysmenorrhea. Conversely, compared with the EMS group without dysmenorrhea, the abundance of *uncultured_Bacteroidales_bacterium* in the EMS group with dysmenorrhea significantly increased (Fig. 2B).

To identify core microbial alterations specifically associated with dysmenorrhea in EMS, "Control vs. EMS+Pain" and "EMS–Pain vs. EMS+Pain" were compared, and the differential microorganisms shared in both comparisons were identified ($p < 0.05$; detailed *p*-values for each comparison are provided in **Supplementary Table 1**). At the genus level, there were 15 commonly upregulated and 10 downregulated genera in the EMS group with dysmenorrhea, including the downregulation of *Morganella* and *Streptomyces* and upregulation of *Clostrid-*

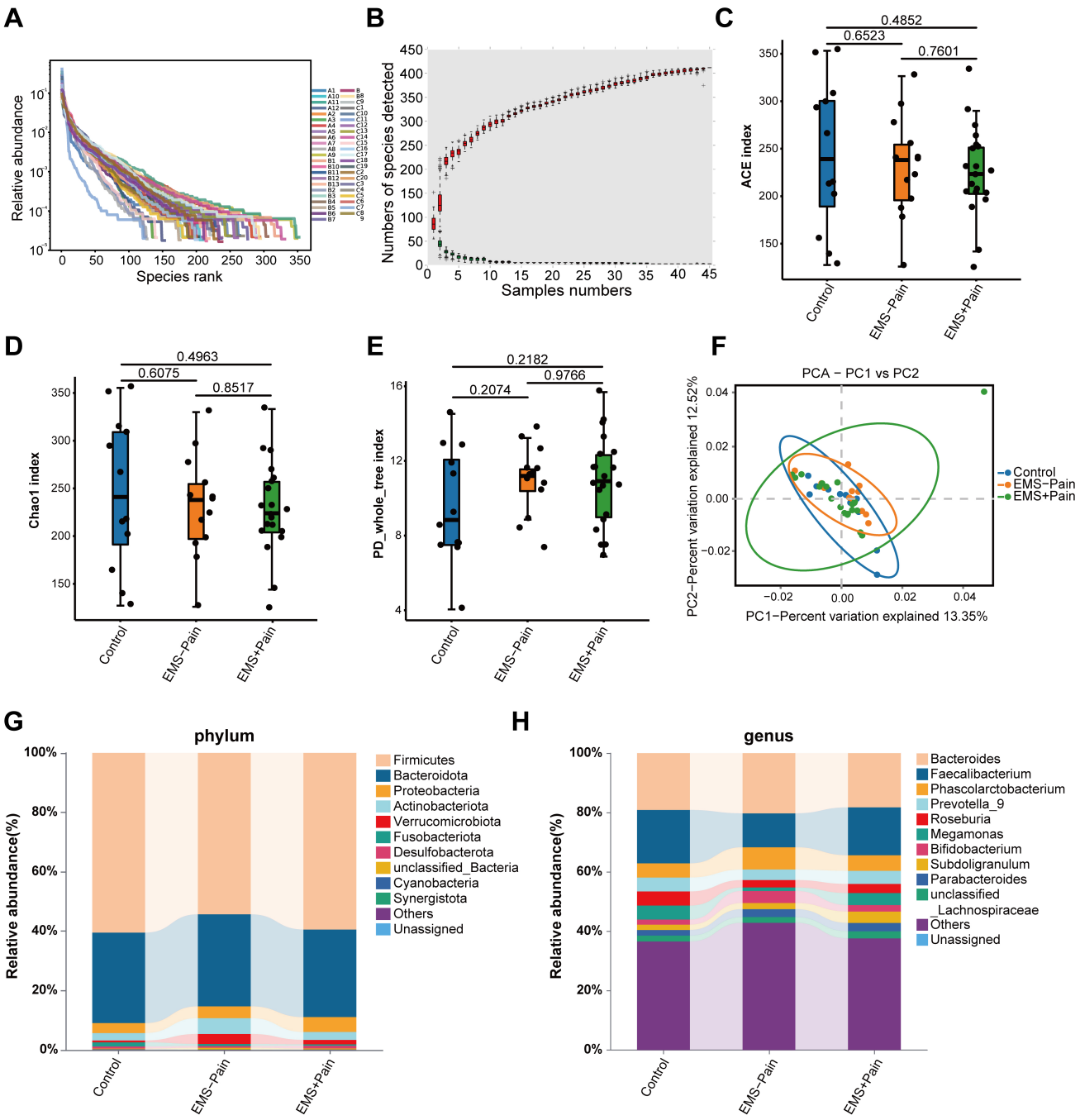

Fig. 1. Gut microbial diversity and structural analysis. (A) Rank–abundance curves. (B) Species accumulation curves. (C) Abundance-based coverage estimator (ACE) indexes. (D) Chao1 indexes. (E) PD_whole_tree indexes. (F) Principal component analysis (PCA) score plot based on the relative abundance of operational taxonomic units (97% similarity). (G) Phylum-level abundance ranking. (H) Genus-level abundance ranking. Control, healthy control group; EMS–Pain, EMS group without dysmenorrhea; EMS+Pain, EMS group with dysmenorrhea.

ioides in the EMS group with dysmenorrhea. At the species level, 27 species were upregulated, and 16 species were commonly downregulated in the EMS group with dysmenorrhea, such as downregulation of *Alistipes inops* and *Gemmobacter aquaticus* and upregulation of *Parabacteroides faecis* among these groups (Fig. 2C). No consistent phylum-level regulation was observed. Notably, several species had expression changes consistent with their corresponding genera. For example, *unclassified_Candidatus_Solibacter*, a species upregulated in the EMS group with dysmenorrhea, belongs to the genus *Candidatus_Solibacter*, which was also upregulated at the genus level. Similarly, *unclassified_Streptomyces* was upregulated, aligning with the upregulation of *Streptomyces* at the genus level. Likewise, *un-*

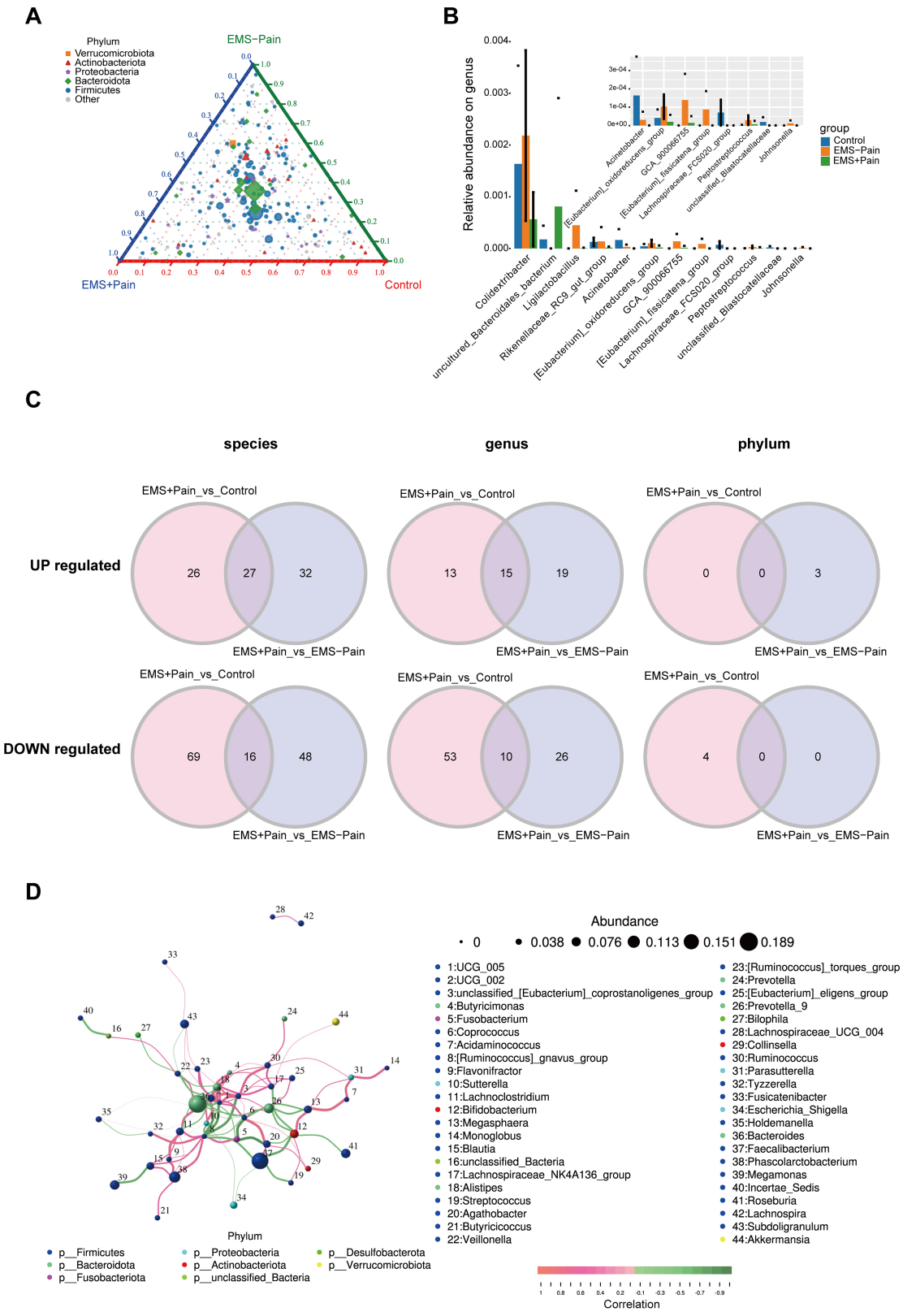

Fig. 2. Screening for differential microorganisms in EMS with pain. (A) Ternary plot showing phylum-level abundance distributions. Pie charts represent phyla, with the size indicating relative abundance; the distance from the vertex reflects group-specific ratio. (B) Relative abundance of the top 12 genera with the smallest *p*-values. (C) Differential microbes at the phylum, genus, and species levels. (D) Correlation analysis of gut microbiota in the EMS group. The dot size indicates relative abundance, and the line color denotes correlation: red for positive and green for negative. Control, healthy control group; EMS–Pain, EMS group without dysmenorrhea; EMS+Pain, EMS group with dysmenorrhea.

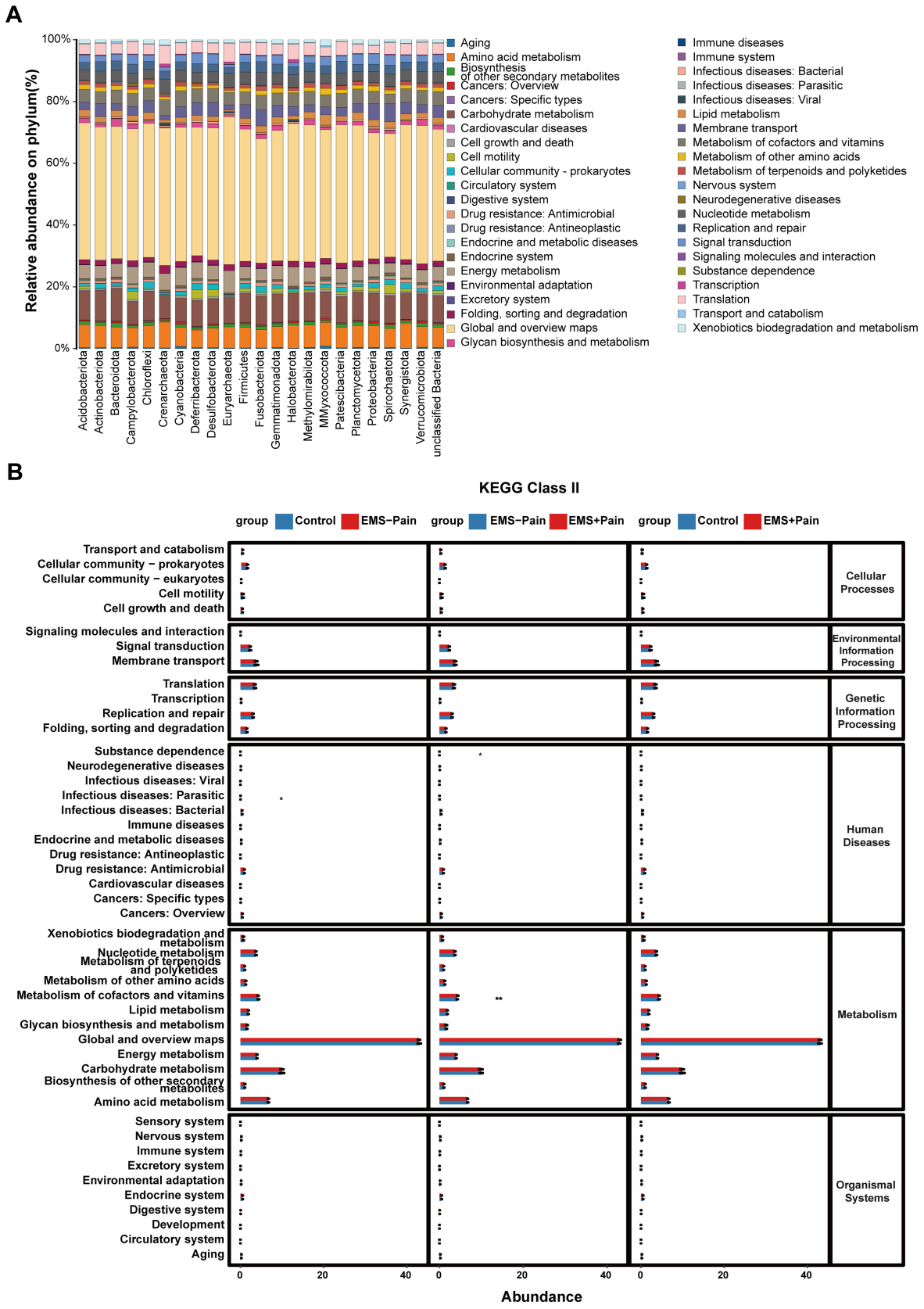

Fig. 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway predictive analysis. (A) KEGG pathway prediction analysis at the phylum level. (B) KEGG pathway prediction analysis among the groups. Control, healthy control group; EMS–Pain, EMS group without dysmenorrhea; EMS+Pain, EMS group with dysmenorrhea. * means $p < 0.05$, ** means $p < 0.01$.

classified_Acinetobacter and *Faecalicoccus pleomorphus* were downregulated at both species and genus levels. These microbes with concordant regulation across taxonomic levels may be crucial in the pathophysiology of EMS-related dysmenorrhea.

Additionally, Spearman's rank correlation analysis of the top 50 genera revealed a robust co-occurrence network, with *UCG_005* and *UCG_002* as the central hub taxa (Fig. 2D). The high abundance levels of *Bacteroides* and *Faecalibacterium* highlighted their potential regulatory roles in EMS-related phenotypes. Collectively, these exploratory findings implicate multilevel gut microbiota alterations in EMS-related dysmenorrhea, though mechanistic roles must be experimentally validated.

3.4 Functional Predictive Analyses of the Gut Microbiota

Community-wide gut microbiota functions were predicted using PICRUSt2 based on clusters of orthologous groups (COG) family data to explore potential roles in EMS and associated pain. Analysis revealed significant functional enrichment across 24 distinct COG categories, particularly in signal transduction mechanisms and amino acid transport and metabolism (**Supplementary Fig. 2A**). Furthermore, intergroup comparisons of COG functional profiles exhibited trends of variation in predicted functions; however, no significant differences were observed (**Supplementary Fig. 2B**).

Gut microbiota-derived KEGG pathway analysis highlighted enrichment in pathways related to amino acid metabolism, metabolism of cofactors and vitamins, signal transduction, and endocrine and metabolic diseases (Fig. 3A). Pairwise KEGG pathway comparisons among the three groups revealed significantly higher enrichment in pathways related to cofactor and vitamin metabolism ($p < 0.01$) and substance dependence ($p < 0.05$) in the EMS group with dysmenorrhea compared with the EMS group without dysmenorrhea (Fig. 3B). Collectively, these predictive functional analyses suggest that the gut microbiota in EMS may modulate amino acid and cofactor metabolism pathways with potential implications for pain mechanisms.

3.5 Correlation Analysis of Differential Microorganisms at Different Taxonomic Levels With Clinical Indicators

To investigate the relationship between differential gut microbes in EMS and clinical indicators of pain and inflammation, correlation analyses were conducted across phylum, genus, and species levels. Clinical parameters included age, BMI, pain severity, AAM, VOCC, AMH, CA125, CA19-9, and NLR.

At the phylum level, *Spirochaetota* and *Deferribacterota* showed negative and positive correlations with NLR, respectively, in the comparison between the healthy control group and EMS group without dysmenorrhea (Fig. 4A). In the comparison between the EMS group without dysmenorrhea and the group with dysmenorrhea, *Crenarchaeota* and *Cyanobacteria* showed significant positive correlations with pain severity (Fig. 4B). Conversely, in the comparison between the healthy control group and the EMS group with dysmenorrhea, *Spirochaetota*, *Deferribacterota*, and *Halobacterota* were negatively associated with pain severity (Fig. 4C).

At the genus level, *Gordonibacter* and *Luteolibacter* showed a negative correlation with NLR, whereas *Mucispirillum* and *Butyricimonas* demonstrated a positive correlation in the comparison of the healthy control group with the EMS group without dysmenorrhea (Fig. 4D). In the analysis between the EMS group without dysmenorrhea and the dysmenorrhea group, *Colidextribacter*, *Faecalicoccus*, and *Acinetobacter* were significantly negatively correlated with the pain severity of dysmenorrhea in EMS. *Oscillibacter*, *Streptomyces,* and *Candidatus_Solibacter* exhibited a significant positive correlation with the degree of pain in EMS (Fig. 4E). In the analysis between the healthy control group and the EMS group with dysmenorrhea, *Acinetobacter* and *Faecalicoccus* showed a significant negative correlation with the degree of dysmenorrhea of EMS, whereas *Candidatus_Solibacter* and *Streptomyces* demonstrated a positive correlation (Fig. 4F).

At the species level, in the comparison between the healthy control group and the EMS group without dysmenorrhea, *Limosilactobacillus_mucosae* and *Bacteroides sp.* showed a negative correlation with NLR, whereas *Megamonas funiformis* and *unclassified_Prevotella* demonstrated a positive correlation with NLR (Fig. 4G). In the analysis between the EMS group without dysmenorrhea and the dysmenorrhea group, *Faecalicoccus_pleomorphus* showed a negative correlation with pain, whereas *unclassified_Candidatus_Solibacter* and *unclassified_Streptomyces* showed a positive correlation with pain (Fig. 4H). In the analysis between the healthy control group and the EMS group with dysmenorrhea, *unclassified_Acinetobacter* and *Faecalicoccus_pleomorphus* displayed a negative correlation with pain, whereas *Alistipes_onderdonkii*, *Alistipes_indistinctus*, and *unclassified_Streptomyces* showed a positive correlation with pain (Fig. 4I). These findings identify microbiota–clinical indicator correlations that indicate potential roles of specific taxa in EMS-related inflammation and pain.

3.6 ROC Analysis of Differential Microorganisms as Diagnostic Markers for EMS

To validate discriminatory microbial markers, ROC curve analysis was performed using an area under the curve (AUC) >0.7 threshold. At the genus level, *Ligilactobacillus* (AUC = 0.7308, sensitivity = 46.15%, specificity = 100.00%) and *Butyricimonas* (AUC = 0.7212, sensitivity = 61.54%, specificity = 91.67%) effectively distinguished the EMS group from the healthy control group (Fig. 5A). Additionally, four genera demonstrated strong discriminatory ability between the EMS group with and with-

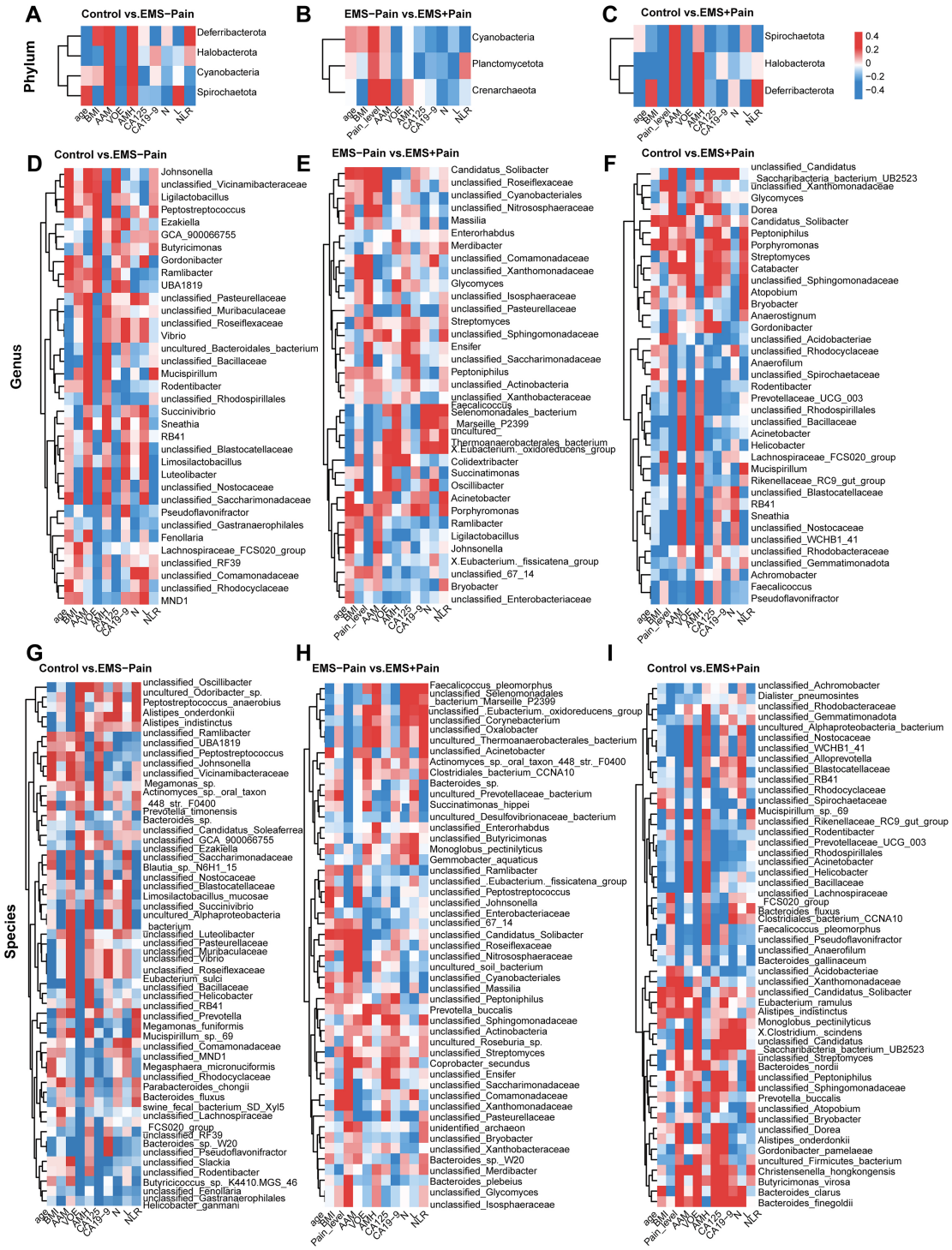

Fig. 4. Correlation analysis of differential microorganisms at different levels of classification with clinical indicators. (A–C) Phylum level: EMS–Pain vs. control and EMS+Pain vs. EMS–Pain, and EMS+Pain vs. control. (D–F) Genus level: EMS–Pain vs. control, EMS+Pain vs. EMS–Pain, and EMS+Pain vs. control. (G–I) Species level: EMS–Pain vs. control, EMS+Pain vs. EMS–Pain, and EMS+Pain vs. control. Control, healthy control group; EMS–Pain, EMS group without dysmenorrhea; EMS+Pain, EMS group with dysmenorrhea.

out dysmenorrhea: *unclassified_Cyanobacteriales* (AUC = 0.7269, sensitivity = 65.00%, specificity = 76.92%), *Oscillibacter* (AUC = 0.7154, sensitivity = 90.00%, specificity = 46.15%), *[Eubacterium]_oxidoreducens_group* (AUC = 0.7462, sensitivity = 90.00%, specificity = 61.54%), and *Colidextribacter* (AUC = 0.7577, sensitivity = 75.00%, specificity = 76.92%) (Fig. 5B). At the species level, *Alistipes_onderdonkii* (AUC = 0.7276,

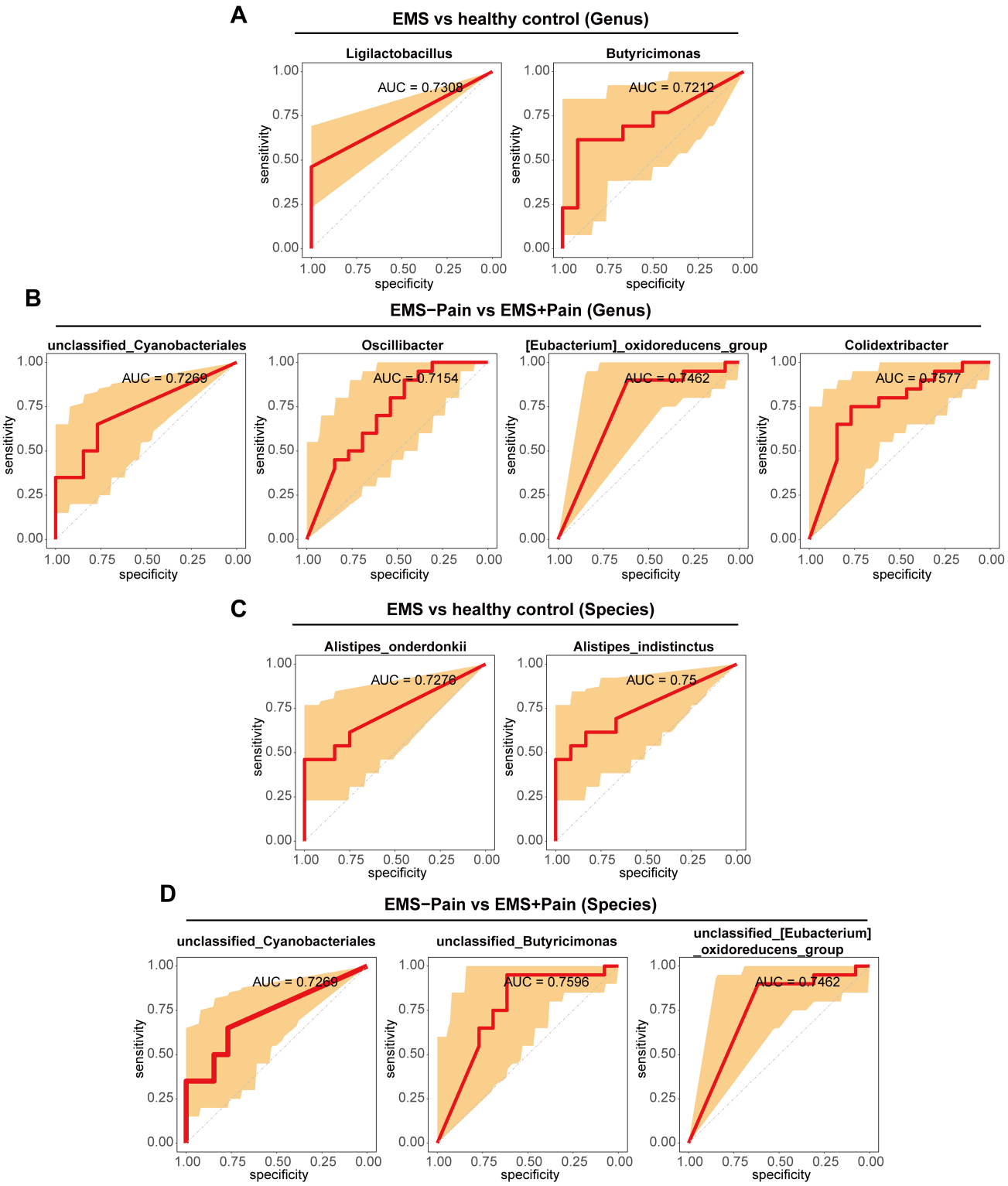

Fig. 5. ROC analysis of differential microorganisms. (A) ROC analysis of differential microorganisms at the genus level between the EMS group and the healthy control group. (B) ROC analysis of differential microorganisms at the genus level between the EMS group with dysmenorrhea and the EMS group without dysmenorrhea. (C) ROC analysis of differential microorganisms at the species level between the EMS group and the healthy control group. (D) ROC analysis of differential microorganisms at the species level between the EMS group with dysmenorrhea and the EMS group without dysmenorrhea. ROC, receiver operating characteristic; Control; healthy control group; EMS–Pain, EMS group without dysmenorrhea; EMS+Pain, EMS group with dysmenorrhea.

sensitivity = 46.15%, specificity = 100.00%) and *Alistipes_indistinctus* (AUC = 0.75, sensitivity = 46.15%, specificity = 100.00%) showed excellent ability to differentiate the EMS group from the healthy control group (Fig. 5C). Similarly, *unclassified_Cyanobacteriales* (AUC = 0.7269, sensitivity = 65.00%, specificity = 76.92%), *unclassified_Butyricimonas* (AUC = 0.7596, sensitivity = 95.00%, specificity = 61.54%), and *unclassified_[Eubacterium]_oxidoreducens_group* (AUC = 0.7462, sensitivity = 90.00%, specificity = 61.54%) discriminated between the EMS groups with and without dysmenorrhea (Fig. 5D). Collectively, these findings highlight *Colidextribacter* and *unclassified_Butyricimonas* as potential microbial markers with robust discriminatory power for EMS-associated dysmenorrhea.

4. Discussion

EMS is a complex chronic inflammatory disease that affects 10%–15% of women of childbearing age [22]. It is characterized by a series of painful symptoms, particularly dysmenorrhea. In recent years, scientific researchers have shown great interest in the interrelationship between the gut microbiota and human health. A study found that the gut microbial composition was disrupted in the EMS patients [19]. In the present study, although no significant differences were observed in the overall microbial diversity or community structure among the healthy control group, EMS group without dysmenorrhea, and the EMS group with dysmenorrhea, specific taxa that exhibited significant differences in relative abundance were identified between the groups. Additionally, several of these differential microorganisms showed correlation with clinical parameters, such as CA125 levels. Moreover, correlations were established between these differential microorganisms and clinical indicators in the EMS group, and specific microorganisms with high predictive value for EMS and EMS pain symptoms were identified.

Previous studies have identified early menarche as a risk factor for both EMS and the severity of dysmenorrhea. A recent cohort analysis indicated that early menarche (<12 years) is significantly associated with increased EMS risk [22], and this association was further confirmed by a meta-analysis of literature published between 2000 and 2020 [23]. Additionally, an earlier menarche onset (<14 years) was linked to increased severity of dysmenorrhea [24]. However, in the present cohort, no significant differences in AAM or BMI were observed among the healthy control group, EMS group without dysmenorrhea, and EMS group with dysmenorrhea. This inconsistency may be attributed to the relatively small sample size and limited statistical power, which could obscure subtle group differences. Moreover, population-specific factors, such as genetic background, lifestyle, or environmental exposures, might modulate these associations in different cohorts. In contrast, serum CA125 levels were significantly higher in the EMS group with dysmenorrhea compared than in the EMS group without dysmenorrhea and the healthy control group, suggesting a more robust association with disease severity and pain phenotype. This increase supports the role of CA125 as a clinically relevant biomarker in EMS, particularly in the context of pain symptoms. Consistent with previous reports, the EMS patients with serum CA125 levels >35 U/mL was found to be more likely to experience dysmenorrhea [25]. These findings highlight the potential utility of CA125 not only in diagnosing EMS but also in stratifying patients based on symptom burden.

Notably, fecal samples from patients with EMS exhibited significant gut microbial dysbiosis, characterized particularly by an altered Firmicutes/Bacteroidetes ratio and increased Bacteroides abundance [26]. In this study, although no significant overall differences were found in microbial community structure, a higher relative abundance of Proteobacteria was observed in the EMS group with dysmenorrhea than in the other groups. Proteobacteria, one of the most common phyla in the human microbiome, is closely related to various inflammatory conditions [27]. γ-Proteobacteria belonging to Proteobacteria were found to be related to inflammatory diseases, such as bowel disease [28]. At the genus level, the relative abundances of Subdoligranulum and Parabacteroides were increased in the EMS group with dysmenorrhea. Shan *et al.* [7] reported that Subdoligranulum abundance was negatively correlated with interleukin-8 (IL-8) levels in patients with EMS, suggesting a potential role in immunomodulation and inflammatory regulation. Moreover, increased levels of Subdoligranulum were also observed in the gut microbiota of patients with unstable angina compared with healthy controls [29], implicating its association with pain conditions. In contrast, the relative abundance of Roseburia in the EMS group was lower than that in the healthy control group. Roseburia is a probiotic that inhibits inflammatory responses in the intestine by producing butyrate in the colon [30]. These findings indicate that EMS and its accompanying dysmenorrhea symptoms may be related to specific changes in the intestinal microbial community.

Accumulating evidence presents that the gut microbiota is involved in the pathogenesis of EMS through mechanisms such as immune regulation, estrogen metabolism, and inflammatory signaling [31]. Among these, metabolite-mediated immune regulation has gained particular attention. Short-chain fatty acids (SCFAs), particularly butyrate, are critical microbial metabolites that influence host physiology, and several studies have highlighted their roles in EMS onset and progression. In line with this, this study identified Roseburia and Butyricimonas, both of which are established SCFA producers, including butyrate and propionate [32,33]. Mechanistic studies have provided further support for this link; for example, Xu *et al.* [34] demonstrated that microbiota-derived acetate ameliorates EMS by modulating M1 macrophage polarization through the

JAK1/STAT3 pathway, whereas Gou *et al*. [35] reported that butyrate enhances ferroptosis sensitivity in EMS via FFAR2/PPAR-γ/PINK1/Parkin-mediated mitophagy. Beyond their role in immune regulation, SCFAs have also been implicated in chronic pain modulation [36], raising the likelihood that altered SCFA production may contribute to EMS-associated dysmenorrhea. Taken together, these findings recommend that specific microbial taxa may influence EMS progression and pain symptoms through metabolite-driven pathways. Together, these findings support the hypothesis that specific gut microbial taxa may influence the pathogenesis of EMS and the occurrence of dysmenorrhea through metabolite-mediated pathways, including SCFA production and immune regulation. Nonetheless, integrated metabolomics-based studies are warranted to further validate these interactions.

At the functional level, COG analysis shows that the COG categories of the three groups of samples were mainly concentrated in fields, such as signal transduction mechanisms and amino acid transport and metabolism. A previous study showed that the balance of the progesterone- and estrogen-related signaling pathways is disrupted in EMS and that this imbalance can worsen inflammation and potentially increased pain from the disease [37]. KEGG pathway prediction showed that EMS was related to pathways, such as the immune system, amino acid metabolism, and cofactor and vitamin metabolism. Notably, vitamin B are critical in maintaining normal neural function, and vitamin B deficiencies have been linked to neuropathic pain symptoms [38]. Recent studies have also reported that vitamin B5 acts as a context-dependent dietary regulator of nociception [39], whereas altered vitamin D status contributes to chronic inflammatory pain in fibromyalgia [40]. These findings advise that alterations in the functional capacity of the gut microbiota may constitute mechanistic links between gut dysbiosis and EMS-associated dysmenorrhea, thereby offering potential targets for therapeutic intervention.

This study identified four microorganisms with predictive value for EMS and seven microorganisms with predictive value for EMS accompanied by dysmenorrhea. Among them, Alistipes_onderdonkii and Butyricimonas had excellent predictive ability for EMS. Studies have shown that oral administration of the commensal bacterium Alistipes onderdonkii can prolong the survival time of allografts and can be used as a probiotic for transplant recipients to reduce inflammation in a stable state following transplantation [41]. Butyricimonas has shown negative correlation with inflammation parameters [42].

These findings have several potential clinical implications. Specific gut microbial taxa and their metabolites may serve as noninvasive biomarkers for diagnosing EMS and distinguishing patients with dysmenorrhea. Moreover, the microbiota–metabolite axis represents a promising therapeutic target, with potential interventions, such as probiotics, dietary modulation, or supplementation with key microbial metabolites such as SCFAs. These strategies could complement existing hormonal or surgical therapies, potentially mitigating disease progression and alleviating pain. Further multi-omics and longitudinal studies may validate these translational applications and assess their clinical feasibility.

Although laparoscopy with histological confirmation remains the gold standard for EMS diagnosis, the exclusive inclusion of patients with imaging-confirmed ovarian endometriomas provided robust diagnostic certainty in our cohort. Transvaginal ultrasonography and MRI demonstrate pathognomonic features for ovarian endometriomas, supporting the reliability of the imaging-based diagnosis in this study. Despite these promising findings, several limitations should be acknowledged. First, the relatively small sample size may limit statistical power and generalizability. Second, the lack of metagenomic or metabolomic data constrains the depth of functional interpretation. Third, the cross-sectional design precludes causal inference between microbial alterations and EMS or its pain-related symptoms. Additionally, the ROC analysis was based on the presence or absence of specific taxa, with presence defined as a relative abundance above a minimal detection threshold to reduce sequencing noise. Future studies incorporating larger cohorts, longitudinal sampling, and multi-omics approaches are warranted to validate these results and further elucidate microbe–host interactions in EMS pathogenesis.

5. Conclusions

This study reveals that alterations in specific microbial taxa, rather than changes in the overall community composition or diversity, are associated with EMS-related dysmenorrhea. Functional prediction analyses showed enrichment in cofactor and vitamin metabolism pathways in EMS-associated pain. The abundances of Acinetobacter and Colidextribacter showed a negative correlation with pain severity. Moreover, 10 microbial species were identified, including Colidextribacter, with moderate diagnostic value (AUC >0.7) for distinguishing the EMS group with dysmenorrhea. These findings highlight potential microbial biomarkers and metabolic pathways for EMS–Pain; however, validation in larger cohorts and mechanistic studies is needed.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

WS, MYW, and SQC conceived and designed the experiments. HLL and XCC conducted the collection and processing of sequencing samples and performed bioinformatics analysis. ZJ and QYZ analyzed the results with the

assistance of XL. WS and MYW authored the manuscript, with revisions provided by SQC and XL. XL, JYM, and LBQ contributed to data interpretation, manuscript drafting, and critical review of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted in accordance with the Declaration of Helsinki. The research protocol was approved by the Ethics Committee of The Sixth Affiliated Hospital of Sun Yat-sen University (2024ZSLYEC-020), and all of the participants provided signed informed consent.

Acknowledgment

We are grateful to the anonymous reviewers for their constructive critiques and insightful recommendations. We also acknowledge the helpful input from our colleagues during the preparation of this paper.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG45531.

References

[1] Bulun SE. Endometriosis. The New England Journal of Medicine. 2009; 360: 268–279. https://doi.org/10.1056/NEJMra0804690.

[2] Crump J, Suker A, White L. Endometriosis: A review of recent evidence and guidelines. Australian Journal of General Practice. 2024; 53: 11–18. https://doi.org/10.31128/AJGP/04-23-6805.

[3] Harel Z. Dysmenorrhea in adolescents and young adults: etiology and management. Journal of Pediatric and Adolescent Gynecology. 2006; 19: 363–371. https://doi.org/10.1016/j.jpag.2006.09.001.

[4] McKenna KA, Fogleman CD. Dysmenorrhea. American Family Physician. 2021; 104: 164–170.

[5] Harel Z. Dysmenorrhea in adolescents and young adults: an update on pharmacological treatments and management strategies. Expert Opinion on Pharmacotherapy. 2012; 13: 2157–2170. https://doi.org/10.1517/14656566.2012.725045.

[6] Nagy H, Carlson K, Khan MAB. Dysmenorrhea. StatPearls Publishing LLC.: Treasure Island (FL). 2025.

[7] Shan J, Ni Z, Cheng W, Zhou L, Zhai D, Sun S, *et al*. Gut microbiota imbalance and its correlations with hormone and inflammatory factors in patients with stage 3/4 endometriosis. Archives of Gynecology and Obstetrics. 2021; 304: 1363–1373. https://doi.org/10.1007/s00404-021-06057-z.

[8] Talwar C, Singh V, Kommagani R. The gut microbiota: a double-edged sword in endometriosis†. Biology of Reproduction. 2022; 107: 881–901. https://doi.org/10.1093/biolre/ioac147.

[9] Guo C, Zhang C. Role of the gut microbiota in the pathogenesis of endometriosis: a review. Frontiers in Microbiology. 2024; 15: 1363455. https://doi.org/10.3389/fmicb.2024.1363455.

[10] Uzuner C, Mak J, El-Assaad F, Condous G. The bidirectional relationship between endometriosis and microbiome. Frontiers in Endocrinology. 2023; 14: 1110824. https://doi.org/10.3389/fendo.2023.1110824.

[11] Lou L, Zhou L, Wang Y. Gut Microbiota: A Modulator and Therapeutic Target for Chronic Pain. Molecular Neurobiology. 2025; 62: 5875–5890. https://doi.org/10.1007/s12035-024-04663-x.

[12] Sugihara N, Okada Y, Tomioka A, Ito S, Tanemoto R, Nishii S, *et al*. Probiotic Yeast from Miso Ameliorates Stress-Induced Visceral Hypersensitivity by Modulating the Gut Microbiota in a Rat Model of Irritable Bowel Syndrome. Gut and Liver. 2024; 18: 465–475. https://doi.org/10.5009/gnl220100.

[13] Jiang Y, Huang Z, Sun W, Huang J, Xu Y, Liao Y, *et al*. Roseburia intestinalis-derived butyrate alleviates neuropathic pain. Cell Host & Microbe. 2025; 33: 104–118.e7. https://doi.org/10.1016/j.chom.2024.11.013.

[14] Wang L, Li T, Cao WX, Zhao JY, Xu XH, Chai JP, *et al*. To explore the mechanism of acupoint application in the treatment of primary dysmenorrhea by 16S rDNA sequencing and metabolomics. Frontiers in Endocrinology. 2024; 15: 1397402. https://doi.org/10.3389/fendo.2024.1397402.

[15] Allaire C, Bedaiwy MA, Yong PJ. Diagnosis and management of endometriosis. CMAJ: Canadian Medical Association Journal = Journal De L'Association Medicale Canadienne. 2023; 195: E363–E371. https://doi.org/10.1503/cmaj.220637.

[16] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics (Oxford, England). 2014; 30: 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

[17] Martin M. Cut adapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal. 2011; 17: 10–12. https://doi.org/10.14806/ej.17.1.200.

[18] Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods. 2013; 10: 996–998. https://doi.org/10.1038/nmeth.2604.

[19] Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics (Oxford, England). 2011; 27: 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

[20] Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, *et al*. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research. 2013; 41: D590–6. https://doi.org/10.1093/nar/gks1219.

[21] Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, *et al*. Author Correction: Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019; 37: 1091. https://doi.org/10.1038/s41587-019-0252-6.

[22] As-Sanie S, Mackenzie SC, Morrison L, Schrepf A, Zondervan KT, Horne AW, *et al*. Endometriosis: A Review. JAMA. 2025; 334: 64–78. https://doi.org/10.1001/jama.2025.2975.

[23] Lu MY, Niu JL, Liu B. The risk of endometriosis by early menarche is recently increased: a meta-analysis of literature published from 2000 to 2020. Archives of Gynecology and Obstetrics. 2023; 307: 59–69. https://doi.org/10.1007/s00404-022-06541-0.

[24] Ullah A, Fayyaz K, Javed U, Usman M, Malik R, Arif N, *et al*. Prevalence of Dysmenorrhea and Determinants of Pain Intensity Among University-Age Women. Pain Medicine (Malden,

Mass.). 2021; 22: 2851–2862. https://doi.org/10.1093/pm/pnab273.

[25] Tang Y, Wen MB, Xiang RM, Yang MT, Shu B, Xu F, *et al*. Serum CA125 as a biomarker for dysmenorrhea in adenomyosis. International Journal of Gynaecology and Obstetrics: the Official Organ of the International Federation of Gynaecology and Obstetrics. 2023; 163: 131–139. https://doi.org/10.1002/ijgo.14832.

[26] Bauşic AIG, Scurtu F, Manu A, Matasariu DR, Brătilă E. Gut Microbiota Dysbiosis in Endometriosis: A Potential Link to Inflammation and Disease Progression. International Journal of Molecular Sciences. 2025; 26: 5144. https://doi.org/10.3390/ijms26115144.

[27] Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends in Biotechnology. 2015; 33: 496–503. https://doi.org/10.1016/j.tibtech.2015.06.011.

[28] Salimi A, Sepehr A, Ajdarkosh H, Aghamohamad S, Talebi M, Rohani M, *et al*. Dynamic Population of Gut Microbiota as an Indicator of Inflammatory Bowel Disease. Iranian Biomedical Journal. 2022; 26: 350–356. https://doi.org/10.52547/ibj.3772.

[29] Liu L, Luo F. Alterations in the fecal microbiota and serum metabolome in unstable angina pectoris patients. Frontiers in Bioscience (Landmark Edition). 2022; 27: 100. https://doi.org/10.31083/j.fbl2703100.

[30] Nie K, Ma K, Luo W, Shen Z, Yang Z, Xiao M, *et al*. *Roseburia intestinalis*: A Beneficial Gut Organism From the Discoveries in Genus and Species. Frontiers in Cellular and Infection Microbiology. 2021; 11: 757718. https://doi.org/10.3389/fcimb.2021.757718.

[31] Moustakli E, Zagorianakou N, Makrydimas S, Oikonomou ED, Miltiadous A, Makrydimas G. The Gut-Endometriosis Axis: Genetic Mechanisms and Public Health Implications. Genes. 2025; 16: 918. https://doi.org/10.3390/genes16080918.

[32] Dicks LMT. How important are fatty acids in human health and can they be used in treating diseases? Gut Microbes. 2024; 16: 2420765. https://doi.org/10.1080/19490976.2024.2420765.

[33] Ma Y, Chen H, Li H, Zheng M, Zuo X, Wang W, *et al*. Intratumor microbiome-derived butyrate promotes lung cancer metastasis. Cell Reports. Medicine. 2024; 5: 101488. https://doi.org/10.1016/j.xcrm.2024.101488.

[34] Xu Y, Zhu Y, Wu X, Peng W, Zhong Y, Cai Y, *et al*. Gut Microbiota-Derived Acetate Ameliorates Endometriosis via JAK1/STAT3-Mediated M1 Macrophage Polarisation. Microbial Biotechnology. 2025; 18: e70202. https://doi.org/10.1111/1751-7915.70202.

[35] Gou Y, Ding J, Wang H, Bai R, Wang H, Wang B, *et al*. Gut microbiota derived butyrate enhances ferroptosis sensitivity in endometriosis through FFAR2/PPAR-γ/PINK1/Parkin mediated mitophagy. Free Radical Biology & Medicine. 2025; 238: 640–652. https://doi.org/10.1016/j.freeradbiomed.2025.07.019.

[36] Tang Y, Du J, Wu H, Wang M, Liu S, Tao F. Potential Therapeutic Effects of Short-Chain Fatty Acids on Chronic Pain. Current Neuropharmacology. 2024; 22: 191–203. https://doi.org/10.2174/1570159X20666220927092016.

[37] Zhang P, Wang G. Progesterone Resistance in Endometriosis: Current Evidence and Putative Mechanisms. International Journal of Molecular Sciences. 2023; 24: 6992. https://doi.org/10.3390/ijms24086992.

[38] Abdelrahman KM, Hackshaw KV. Nutritional Supplements for the Treatment of Neuropathic Pain. Biomedicines. 2021; 9: 674. https://doi.org/10.3390/biomedicines9060674.

[39] Hamoudi Z, Leung C, Khuong TM, Cooney G, Neely GG. Vitamin B5 is a context-dependent dietary regulator of nociception. G3 (Bethesda, Md.). 2024; 14: jkae174. https://doi.org/10.1093/g3journal/jkae174.

[40] Saija C, Bertuccio MP, Scoglio A, Macaione V, Cacciola F, Micalizzi G, *et al*. Role of Vitamin D Status and Alterations in Gut Microbiota Metabolism in Fibromyalgia-Associated Chronic Inflammatory Pain. Biomedicines. 2025; 13: 139. https://doi.org/10.3390/biomedicines13010139.

[41] Li Z, Rasic M, Kwan M, Sepulveda M, McIntosh C, Shastry V, *et al*. Oral administration of the commensal Alistipes onderdonkii prolongs allograft survival. American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2023; 23: 272–277. https://doi.org/10.1016/j.ajt.2022.11.011.

[42] Xia T, Duan W, Zhang Z, Li S, Zhao Y, Geng B, *et al*. Polyphenol-rich vinegar extract regulates intestinal microbiota and immunity and prevents alcohol-induced inflammation in mice. Food Research International (Ottawa, Ont.). 2021; 140: 110064. https://doi.org/10.1016/j.foodres.2020.110064.