

# Comparative gut microbiome profiling in primary dysmenorrhoea rat models via 16s rRNA gene next generation sequencing

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## ABSTRACT

**Introduction:** Primary Dysmenorrhoea (PD) is a prevalent gynaecological disorder affecting women worldwide. Emerging evidence suggests a bidirectional relationship between the gut microbiome and reproductive health, particularly through the modulation of the estrobolome. However, microbial profiles associated with PD remain poorly characterised, particularly in preclinical models. **Objectives:** This study aimed to characterise the alterations in microbiome profiles between control and PD-induced rat models while evaluating the modulatory effects of mefenamic acid and probiotic treatments in PD model rat group via high-throughput sequencing. **Materials and Methods:** Twenty-eight female Sprague-Dawley rats were divided into four groups (n=7): a control group (Group 1), a PD model group (Group 2), PD group treated with Mefenamic Acid (MA) (Group 3), and PD group treated with Probiotics (Group 4). Gut samples were collected, and DNA was extracted upon euthanasia. Microbiome profiling was conducted by targeting the V3 region of the 16S rRNA gene. Diversity measures were analysed using alpha and beta diversity matrices to uncover microbial disparities between the rat groups. **Results:** This study revealed that alpha diversity analysis indicated an increased shift towards microbial richness in the PD + Probiotics group, as indicated by a higher Shannon index compared to the untreated PD group, though this does not reach statistical significance (p-value = 0.2494). Beta diversity based on PCoA analysis showed a partial separation between the untreated PD group and treatment groups (PD + MA and PD + Probiotics), suggesting treatment-induced shifts in microbial community composition (p-value = 0.12). Remarkably, among the top ten most abundant taxa identified, core microbiome analysis showed that the PD model group was enriched with pro-inflammatory family Helicobacteraceae, and genera *Pseudomonas* sp., *Turicibacter* sp. and *Eubacterium* sp. CAG-274. In contrast, the control group was enriched with a higher relative abundance of beneficial genera such as *Lactobacillus* sp., *Eubacterium* sp., *Bifidobacterium* sp. and *Blautia* sp. – taxa associated with estrobolome and implicated in estrogen metabolism and homeostasis. **Conclusion:** This study reveals differences in microbial diversity between the rat groups, illustrating a possible link between gut microbiome alteration and PD disease mechanism. Ultimately, these findings will open new avenues for microbiome-based therapeutic strategies to alleviate PD symptoms, warranting further research in the future.