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Research article

Escherichia coli extract (OM-89) preserves gut microbial balance more effectively than antibiotics in a healthy mouse model

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ABSTRACT

Urinary tract infections (UTIs) are among the most common infectious diseases globally, with a recurrence rate of approximately 20-30% in patients. Traditionally, preventing recurrent UTIs (rUTIs) often involves the prolonged use of low-dose antibiotics. However, these approaches can lead to gut dysbiosis, a condition associated with various adverse effects and potentially contributing to rUTIs. OM-89 is a non-antibiotic option for preventing rUTIs, but its impact on the gut microbiota is poorly understood. This study compared the effects of OM-89 and the antibiotic ciprofloxacin on the gut microbiota of healthy C57BL/6 mouse model over a four-week period. Fecal microbiota were analyzed using 16s rRNA nanopore long-read sequencing. The findings revealed that OM-89 effectively preserved the overall diversity and balance of the gut microbiome. In contrast, ciprofloxacin treatment resulted in marked disruptions of gut microbial composition, characterized by reductions in richness and evenness, accompanied by increases in the relative abundance of *Akkermansia muciniphila* and *Phocaeicola vulgatus*. This suggests that OM-89, unlike antibiotics, exerts a milder impact on the gut microbiota in healthy mice. Future studies using uropathogenic infection models are essential to determine whether the preservation of gut microbiota by OM-89 directly correlates with the prevention of rUTI recurrence. Furthermore, clinical investigations are warranted to confirm the tangible clinical benefits of these effects in patients. Such investigations will clarify the previously unclear mechanisms of OM-89 from the novel perspective of gut microbial balance, thereby establishing a clinical rationale for rUTI prevention.

1. Background

Urinary Tract Infections (UTIs) are a major global health issue affecting millions of people worldwide. These infections can occur in any part of the urinary tract, including the urethra, bladder, ureters, and kidneys [1,2]. Recurrent UTIs (rUTIs) are defined as two or more occurrences within six months or three or more within one year, presenting a major challenge in clinical management. Approximately 20-30% of individuals who have experienced a UTIs will have a recurrence [3,4]. This high recurrence rate has necessitated the long-term use of low-dose antibiotics to prevent of rUTIs [4]. However, this approach can induce gut dysbiosis, potentially increasing the antibiotic-associated side effects and facilitating the emergence of antibiotic resistance [2,4,5]. Consequently, there is growing interest in non-antibiotic prophylactic approaches to preventing rUTIs.

OM-89 (Uvo) is a bacterial extract containing immunostimulatory components derived from 18 strains of uropathogenic *Escherichia*

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coli (UPEC) [6]. This non-antibiotic therapeutic agent has been shown to reduce the recurrence of UTIs and improve voiding difficulties, while also decreasing leukocyturia and bacteriuria [6–8]. Uvo might function as an immunogen that could promote antibody production and is suggested to induce the development of $CD4^+$ Th1 cells mediated by dendritic cells [9–11]. Despite these promising effects, the exact mechanisms by which Uvo provides its therapeutic benefits remain unclear.

The gut serves as a reservoir for UPEC, which causes urinary tract infections (UTIs), and the gut microbiota is closely associated with UTIs [12,13]. The use of broad-spectrum antibiotics disrupts the gut ecosystem, increasing the risk of recurrence and promoting antibiotic resistance [14]. Additionally, gut dysbiosis has been observed in female patients with rUTIs, along with a reduction in microbial diversity and depletion of butyrate-producing bacteria [15–17]. In both patients suffering from rUTIs and a UTI mouse model, probiotic administration resulted in significant prevention of UTIs and improvement of clinical conditions [18–20]. These findings support the gut-bladder axis concept, which suggests a connection between gut and urinary tract health, highlighting the significant role of gut microbiota in UTI recurrence. However, most previous studies have primarily focused on the direct effects on UPEC, with limited research addressing gut microbiota diversity. Furthermore, the gut microbiota has been shown to be essential for maintaining systemic health and overall well-being, as well as playing a crucial role in drug metabolism and responsiveness [21–23]. Consequently, understanding how pharmaceuticals interact with the gut microbiota has become increasingly important. Although clinical studies suggest that Uvo may effectively prevent rUTIs, research on its effects on gut microbiota remains limited, despite the growing recognition of the importance of gut microbiota in modern medicine. Specifically, no studies have been conducted to evaluate the impact of Uvo on gut microbial diversity in animal or clinical models.

Herein, the first study directly comparing the effects of Uvo and antibiotic ciprofloxacin on the gut microbiota of healthy C57BL/6 mice is presented. By employing Nanopore long-read sequencing, a comprehensive taxonomic analysis was conducted at the species level, providing a detailed characterization of microbial communities. The findings reveal that Uvo influences specific microbial taxa while preserving overall microbial diversity, which starkly contrasts the disruptive effects observed with antibiotics. These results highlight the potential of Uvo as a therapeutic agent that supports gut microbiota stability, underscoring its advantages over conventional antibiotic treatments.

2. Materials and methods

2.1. Animal and experiment design

Eight-week-old C57BL/6 mice were purchased from Orient Bio Inc. They were accommodated under specific pathogen-free (SPF) conditions and allowed a 4-week acclimatization period prior to the start of experimental procedures. Mice had ad libitum access to food and water and were maintained on a 12-h light/dark cycle. All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Yonsei University College of Medicine (IACUC number: 2022 - 0106) and were conducted in accordance with ethical guidelines for the care and use of laboratory animals. At the age of 12 weeks, mice were randomly assigned to one of four groups (n = 8 per group); (1) No treatment group, with no intervention; (2) Placebo group, receiving saline solution containing the same amount of excipients (Magnesium Silicate, Magnesium stearate, Mannitol, Corn starch) as the daily dose given to the Uvo group; (3) Uvo group, receiving a dose of Uvo containing 1 mg of lyophilized extract of uropathogenic *Escherichia coli*, the main ingredient, dissolved in a saline solution, as previously reported in murine studies [24]; (4) Antibiotic group, receiving ciprofloxacin (Sigma-Aldrich, Cat# 17850) at a dose of 2.6mg/head/day, corresponding to the human equivalent dose calculated using body surface area-based allometric scaling. All administrations were performed orally, 5 times a week, with each administration being 0.5 ml, for a duration of 4 weeks. Fecal samples were collected before the start of treatment (baseline) and at the end of the treatment period. Samples were immediately frozen at −80 °C until further analysis.

2.2. Stool DNA extraction

DNA was extracted from stored stool samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Cat# 51604). Briefly, samples were homogenized in 1 ml of InhibitEX buffer by vortexing for 1 min. After centrifugation at full speed for 1 min to remove stool particles, 600 μl of the supernatant was transferred to a new 2 ml collection tube. To this, 25 μl of proteinase K and 600 μl of Buffer AL were added, and the mixture was incubated at 70 °C for 10 min. Subsequently, 600 μl of ethanol (96 – 100 %) was added and the sample was vortexed again. The mixture was then purified through a column, followed by DNA elution using ATE buffer. DNA concentration was measured using a Nanodrop, and samples were stored at temperatures ranging from −15 °C to −30 °C until further experiments.

2.3. 16s rRNA nanopore sequencing

Extracted genomic DNA (gDNA) was processed for PCR amplification and library preparation using the 16S barcoding kit (Oxford Nanopore Technologies, Cat# SQK-16S024). This kit integrates PCR amplification and barcoding into a single process. The PCR amplification was performed using 25 μl of LongAmp Hot Start Taq 2X Master Mix (NEB, Cat# M0533S), 10 μl of input DNA (10 ng), 5 μl of nuclease-free water, and 10 μl of 16S barcode primers. The cycling conditions were set as follows: initial denaturation at 95 °C for 1 min; 25 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, and extension at 65 °C for 2 min; followed by a final extension at 65 °C for 5 min. The produced 16s amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter™, Cat# A63881). The concentration of the purified samples was measured with a Qubit 4.0 fluorometer, and they were diluted to 50-100 fmol

in 10 mM Tris-HCl pH 8.0 with 50 mM NaCl for library loading. To prepare for the flow cell loading, 11 μl of the DNA library, 34 μl of Sequencing Buffer, 25.5 μl of Loading Beads, and 4.5 μl of nuclease-free water were mixed and loaded onto the R9.4.1 flow cell (Oxford Nanopore Technologies, Cat# FLO-MIN106D). Sequencing was conducted using the MinION platform (RRID:SCR_017985).

2.3.1. *Quantification and statistical analysis*

2.3.1.1. Bioinformatic analysis. The sequencing was performed using MinKNOW software (v22.12.7) with guppy (v6.4.6, RRID:SCR_023196) live basecalling. Data with quality scores > Q7 were selected. Adapter trimming was performed using Porechop (v0.2.3, RRID:SCR_016967) to remove barcodes when barcoding was applied. Subsequently, NanoFilt (v2.8.0, RRID:SCR_016966) [25] was used to further filter the data, retaining reads with quality scores ≥ Q10. Quality control assessment was performed with NanoPlot (v1.40.0, RRID:SCR_024128) [26], a plotting tool for long read sequencing data and alignments. Reads were filtered to retain those with lengths between 1000 and 2000 bp, resulting in 5000 reads per sample used for subsequent analyses. Metagenomic analysis was performed using EPI2ME (v2.8.0, RRID:SCR_025280). Alpha diversity (intra-sample diversity) was evaluated using several indices provided by EPI2ME—namely, Richness, Inverse Simpson, Simpson, Shannon, Effective number of species, Pielou's evenness, Fisher's alpha, and the Berger Parker index. Beta diversity, which reflects the variation in diversity between pre-treatment and post-treatment groups, was assessed using the Bray-Curtis dissimilarity index. Based on this metric, the distance matrix was calculated and statistical significance between groups was determined using Permutational Multivariate Analysis of Variance (PERMANOVA) implemented in the vegan package (v2.6-4, RRID:SCR_011950). Principal Coordinates Analysis (PCoA) plots, generated from the Bray-Curtis distance matrix, were employed to visualize the similarities and differences among samples. Furthermore, clustering was performed using the

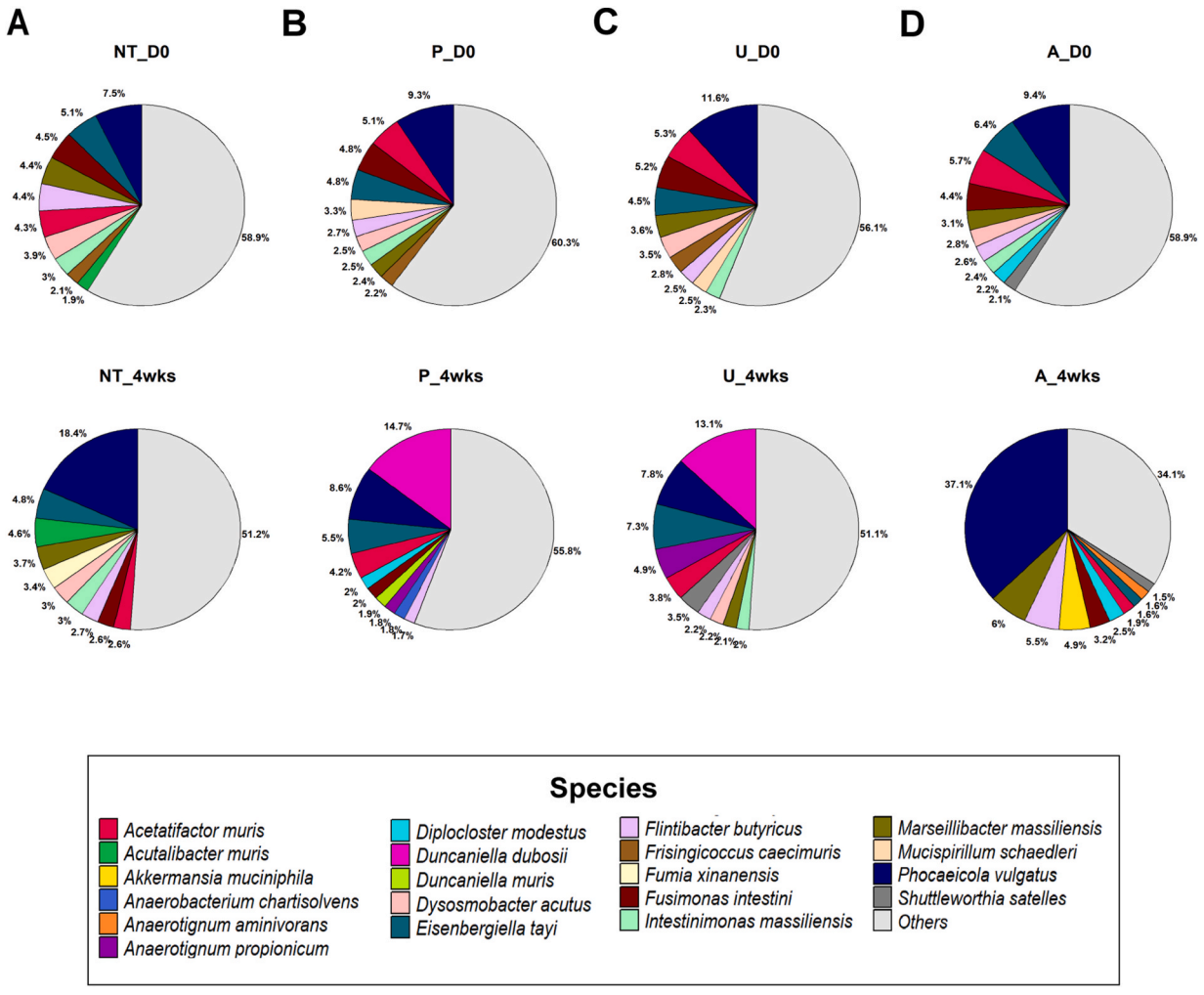

Fig. 1. Changes in gut microbial composition in different treatment groups before and after drug delivery. The relative abundance of bacterial species within each treatment group is shown at two-time points, baseline (D0) and after 4 weeks, with each group consisting of n = 8. Each color represents a specific microorganism, and "others" refers to all species excluding the top 10 in each group. The abbreviations are as follows: NT, no treatment; P, placebo; U, OM-89 (Uvo); A, antibiotics (ciprofloxacin).

unweighted pair group method with arithmetic mean (UPGMA) via the phangorn package (v2.11.1, RRID:SCR_017302) to construct a phylogenetic tree representing the community structures between groups. Differential abundance analysis was conducted using DEseq2 (v1.42.1, RRID:SCR_015687) [27] and Lefser (v1.12.1). These analyses identified significantly differentially abundant taxa between groups.

2.3.1.2. Statistical analysis. All statistical analyses were performed using Python. Data were analyzed using a Wilcoxon signed rank test, with *p*-values adjusted using the Benjamini-Hochberg false discovery rate (FDR) method. Statistical significance was denoted as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. 'ns' indicates a lack of statistical significance.

3. Results

1. Alteration in gut microbial composition following drug administration.

The microbial species distribution at baseline (D0) and after 4 weeks under four conditions; non-treated (NT), placebo (Plc, P), OM-89 (Uvo, U) and ciprofloxacin (Cip, A). At baseline, *Phocaeicola vulgatus* was the predominant species in all groups, with prevalences ranging from 7.5 to 11.6%. It was followed by several species including *Acetatifactor muris*, *Fusimonas intestini*, and *Eisenbergiella tayi*, which exhibited abundances in the range of 1.9 to 6.4%. Species not ranked in the top 10 were categorized as "Others", accounting for

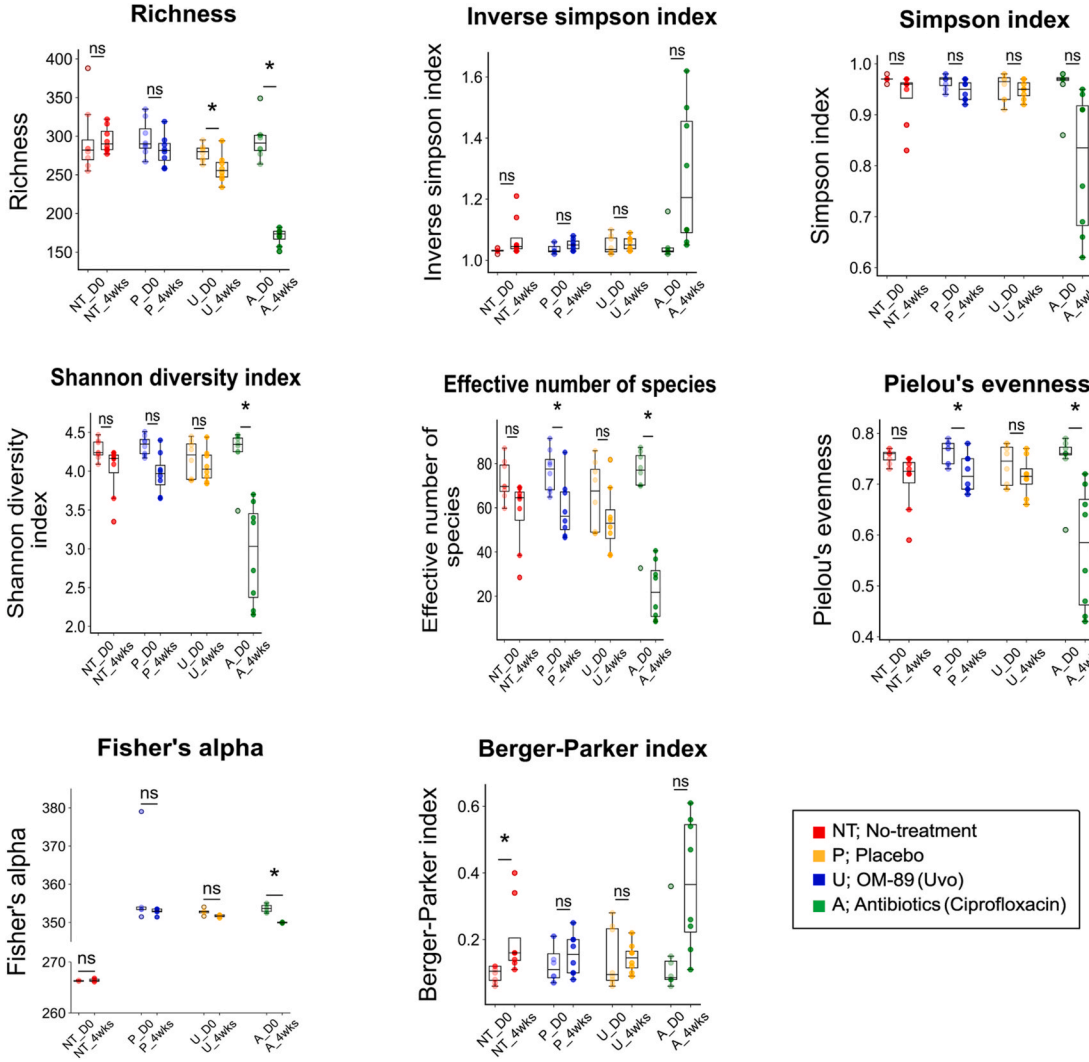

Fig. 2. Comparative analysis of alpha diversity indices among groups before and after drug administration. Each box plot represents the distribution of diversity indices within each group (n = 8 per group), indicating the median, quartiles, and outliers. The differences between groups were analyzed using a Wilcoxon signed rank test, and *p*-values were adjusted using the FDR method of Benjamini and Hochberg. The abbreviations are as follows: NT, no treatment; P, placebo; U, OM-89 (Uvo); A, antibiotic (ciprofloxacin). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

56.1 to 60.3% of the total microbial population (Fig. 1).

After four weeks, in the NT group, the proportion of *Phocaeicola vulgatus* increased to 18.4%, marking the most significant change with approximately a 2.5-fold increase from baseline (Fig. 1a). Compared to the NT group, the other groups showed more pronounced changes in microbial composition. Specifically, in the Plc group, *Duncaniella dubosii* emerged as a new dominant species at 14.7% of the microbial population. Concurrently, *Phocaeicola vulgatus*, *Eisenbergiella tayi* and *Acetatifactor muris* remained prominent. Additionally, species such as *Dilpocloster modestus* and *Duncaniella muris* emerged as new predominant species following the Plc treatment (Fig. 1b).

After four weeks of Uvo treatment, the microbial composition closely mirrored that of the Plc group, with *Duncaniella dubosii* emerging as the dominant species. The continued presence of *Phocaeicola vulgatus* and *Eisenbergiella tayi* among the top species was similar to that in the Plc group. However, Uvo differed from Plc with a higher abundance of *Anaerotignum propionicum* (4.9% versus 1.8%), and the emergence of *Shuttleworthia satelles* as a newly predominant species (Fig. 1c).

After Cip treatment, *Phocaeicola vulgatus* underwent a remarkable four-fold increase to 37.1%, accounting for over a third of the total microbial distribution, and *Akkermansia muciniphila* emerged as a new leading species at 4.9%. Concurrently, the "Others" category significantly declined from 58.9% to 34.1% (Fig. 1d). This suggests that exposure to antibiotics resulted in certain species rising to dominance within the gut microbiota while relative proportions of other species decreased.

2. Uvo preserves alpha diversity contrasting with detrimental effects from ciprofloxacin.

To investigate the impact of drug treatment (Plc, Uvo and Cip) on gut microbial diversity, samples from each group were analyzed for intra-sample diversity both before and after treatment. Various alpha diversity indices were used to measure microbial diversity at these time points, specifically richness, evenness, and dominance. The statistical significance of changes in microbial diversity associated with drug administration was determined using the Wilcoxon signed-rank test.

Species richness remained unchanged before and after treatment in the NT and Plc groups. In contrast, richness in the Uvo group was slightly reduced post-treatment ($p < 0.05$), whereas the Cip group showed a substantial decrease ($p < 0.05$). Fisher's alpha consistently stayed lower in the NT group compared to the other group, but there were no significant changes post-treatment. This indicates that microbial diversity was not significantly impacted by the passage of time throughout the study. The Plc and Uvo groups maintained stable Fisher's alpha values, whereas significant reductions were observed in the Cip groups ($p < 0.05$) (Fig. 2).

The Shannon diversity index decreased significantly in Cip group ($p < 0.05$), whereas no significant changes were observed in the other groups. Metrics associated with Shannon diversity, including Pielou's evenness and Effective number of species, decreased significantly in both the Plc and Cip groups ($p < 0.05$ for both groups). This reduction was particularly pronounced in Cip group compared with the other groups (Fig. 2). Additionally, no significant changes were observed in the Simpson diversity index across any group. The Berger-Parker index increased significantly in the NT group ($p < 0.05$), while no significant differences were detected in the Plc, Uvo, and Cip groups.

Collectively, these findings suggest that the antibiotic Cip significantly compromised gut microbial diversity, reflecting a disruption in the overall balance of the gut microbial community. In comparison, Plc and Uvo demonstrated only a slight tendency toward reduced microbial diversity, but since these changes were also observed in the NT group, it indicates that their impact on microbial diversity was minimal.

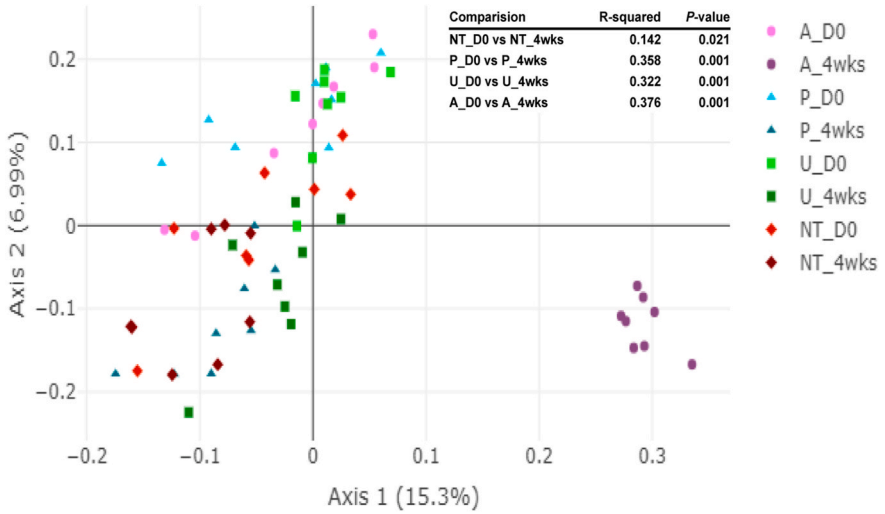

Fig. 3. Beta diversity analysis of gut microbiota following Uvo, Plc, Cip and NT treatment. The PCoA plot of Bray-Curtis dissimilarity illustrates the beta diversity of the gut microbiome before and after treatment with Uvo, Plc, Cip and NT, where symbols and colors denote specific treatment groups and time points. The abbreviations are as follows: NT, no treatment; P, placebo; U, OM-89 (Uvo); A, antibiotic (ciprofloxacin).

3. Uvo and Plc exhibit similar microbial communities.

To evaluate the differences in species composition among groups before and after drug administration, beta diversity analysis was conducted using the Bray-Curtis dissimilarity index, based on the relative abundance of species. The statistical significance of these dissimilarities was assessed via PERMANOVA analysis. PCoA based on Bray-Curtis dissimilarity revealed distinct clustering of the samples along the axis (15.3%) and axis2 (6.99%) post-treatment.

Excluding the Cip group, other groups shifted along axis 2 after four weeks, while Cip group exhibited pronounced movements along both axes 1 and 2 following administration. The NT group exhibited the least variability between baseline and four weeks ($r = 0.142$, $p = 0.021$), maintaining an overall similar cluster structure with slight differences. In contrast, the Plc and Uvo groups initially overlapped in similar regions. After four weeks, although they formed distinct clusters compared to the baseline, they continued to share similar regions. Notably, the Cip group resulted in the most distinct clustering and the highest variability among all the groups ($r = 0.376$, $p = 0.001$) (Fig. 3).

Additionally, UPGMA clustering and heatmap analysis based on Bray-Curtis distances revealed taxonomic differences among microbial communities across the various treatment groups. The analysis identified four major clusters. Cluster 1 included samples obtained four weeks following the administration of Cip, indicating pronounced shifts in microbial composition. Cluster 2 comprised samples collected four weeks after administration of Plc and Uvo, which shared similar microbial communities. This suggests that the active ingredient of Uvo exerts limited influence on the diversity of the gut microbiota, implying that changes associated with Uvo may be driven by other components of the drug. Cluster 3 was composed of mixed samples from the NT group, taken at baseline and four weeks, while Cluster 4 consisted of the remaining NT group and baseline samples from the Plc, Cip, and Uvo groups (Fig. 4).

4. Uvo increased the abundance of beneficial gut bacteria, whereas Cip disrupted various commensal bacteria.

Differential abundance of microbial species before and after drug administration was analyzed using DESeq2 and LEfSe.

The Deseq2 analysis revealed no significant changes in microbial species within the NT group. However, the Plc group displayed significant increases in 50 microbial species, including *Duncaniella spp.*, *Roseburia lenta*, *Muribaculum gordoncarteri*, and *Ruminococcus champanellensis*, following drug administration. Conversely, 19 species such as, *Butyrivibrio proteoclasticus* and *Anaerocolumna cellulosilytica* significantly decreased. The Uvo group exhibited increased in 25 species, including *Duncaniella spp.*, and decreased in 9 species post-treatment (Fig. 5a and b). Among these changing species, 72% of the increased species, including *Duncaniella spp.*, *Ruminococcus albus*, *Parabacteroides goldsteinii*, and *Akkermansia muciniphila*, and 56% of the decreased species, including *Butyrivibrio proteolyticus*, shared similar patterns with the Plc group (Fig. 6). Additionally, the Cip group noted a significant increase in 29 species,

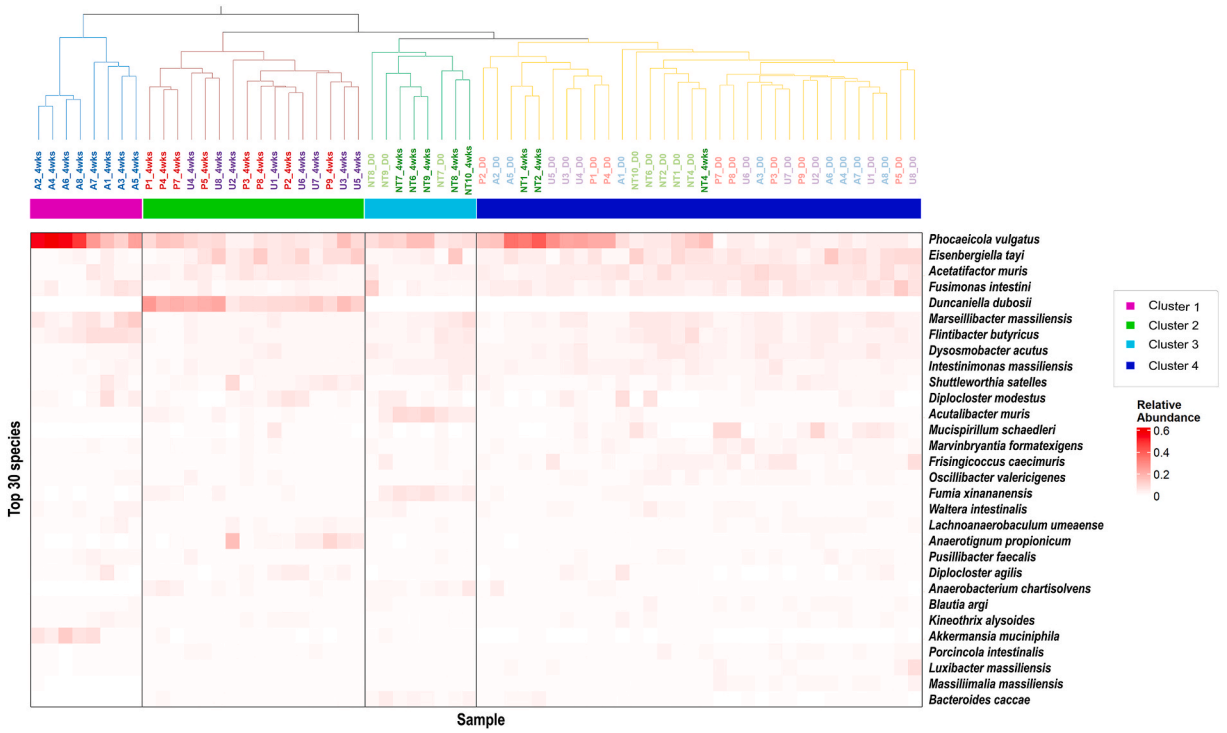

Fig. 4. Microbial Community Similarity and Abundance The UPGMA cluster tree, based on Bray-Curtis dissimilarity, visualizes the similarity of microbial communities among samples, and the accompanying heatmap depicts the relative abundance of the top 30 bacterial species, with darker red indicating higher abundances. The abbreviations are as follows: NT, no treatment; P, placebo; U, OM-89 (Uvo); A, antibiotic (ciprofloxacin).

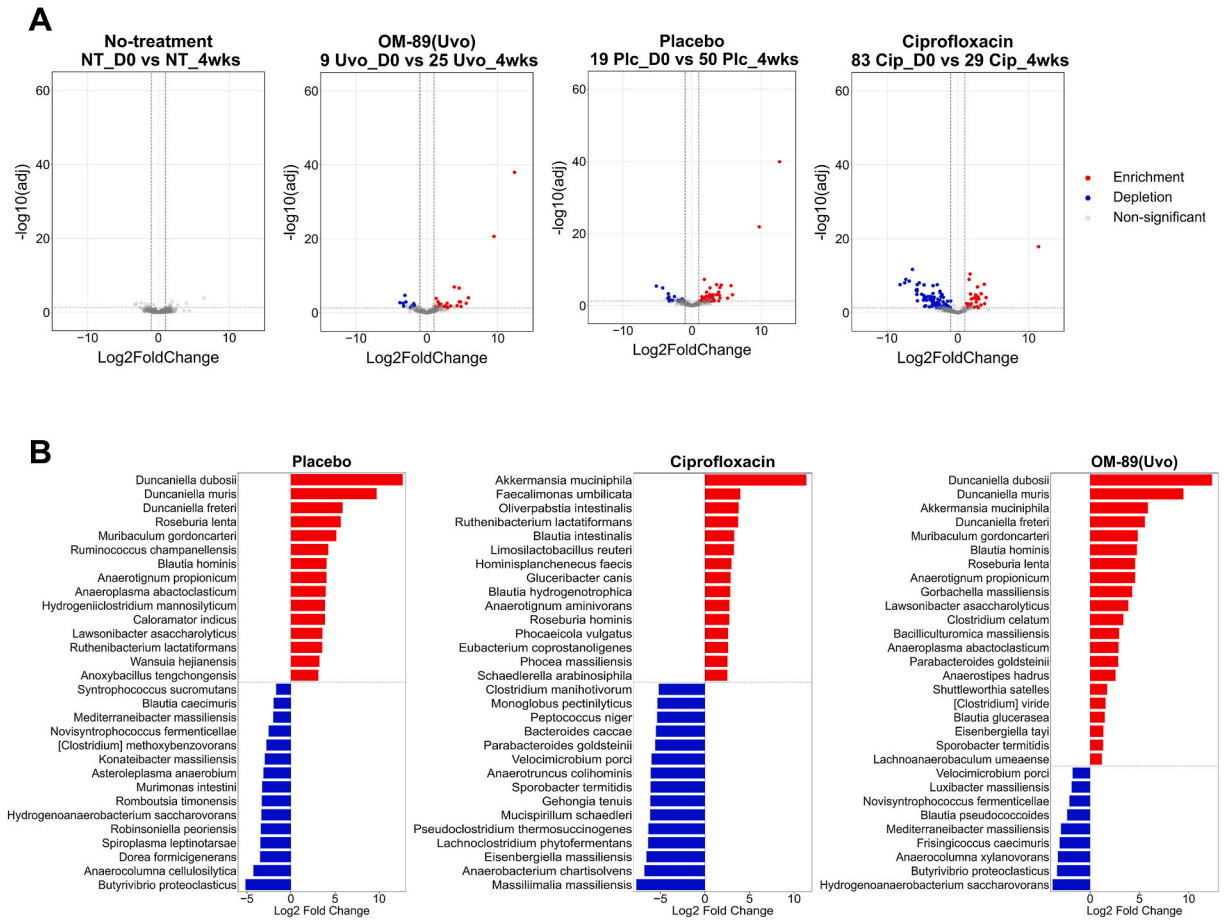

Fig. 5. Changes in microbial species abundance following drug treatment. (A) Differential abundances of microbial species following NT, Plc, Uvo and Cip treatments. Each point represents a microbial species. Species with $|\log FC| \leq 1$ are shown in gray, species with logFC >1 are shown in red (significant enrichment), and species with logFC < -1 are shown in blue (significant depletion). (B) Bar chart representing the representative species with significantly increased or decreased abundance between groups.

with a notable increase in *Akkermansia muciniphila*, while 83 species, including *Massilimalia massiliensis* and *Ruminococcus spp*. Decreased (Fig. 5a and b).

The LEfSe analysis identified an increase in *Phocaeicola vulgatus* (LDA score = 2.4) in the NT group, a result not observed in the Deseq2 analysis. In the Plc group, *Duncaniella dubosii* showed a significant increase, which was also observed in the Uvo group (LDA score = 2.5 for both Plc and Uvo). Additionally, *Anaerotignum propionicum* was enriched in the Uvo group (LDA score = 2.0). This further supports the similarity in microbial impact between Plc and Uvo groups. In the Cip group, increase in *Akkermansia muciniphila* (LDA score = 2.0) and *Phocaeicola vulgatus* (LDA socre = 2.9) were noted, along with decrease in *Eisenbergiella tayi* (LDA score = −2.1) and *Acetatifactor muris* (LDA score = −2.0). This group exhibited a relatively higher number of changes in microbial species compared to the others (Fig. 7a and b).

In conclusion, this study found that Uvo, Plc and Cip treatments were associated with different patterns of change in the gut microbial community. The Uvo and Plc groups showed alterations in some common species. However, the overall extent of these changes appeared to be limited, and microbial diversity remained relatively stable. By contrast, the Cip group exhibited changes in a larger number of species, with a tendency towards reduced diversity and a shift of the gut environment into dysbiosis. These findings suggest that Cip treatment may place greater pressure on the stability of the gut microbiota, whereas Uvo treatment was linked to more modest changes and may help maintain microbial diversity in a relatively stable state.

4. Discussion

This study is the first to evaluate the potential impact of Uvo on gut microbial diversity. The results show that the group administered antibiotics experienced a significant decrease in gut microbial diversity, whereas the group treated with Uvo maintained a more stable microbial community. These findings not only align with the existing understanding that antibiotics disrupt the gut microbial ecosystem, but also demonstrate that Uvo, as a non-antibiotic therapy, may play a positive role in preserving microbial diversity.

Fig. 6. Visualization of microbial species affected by Plc and Uvo treatment using a Venn diagram. Microbial species selected using DESeq2 were visualized through a Venn diagram to illustrate the effects of Plc and Uvo treatments. This diagram distinguishes species uniquely affected by each treatment condition and those influenced by both conditions.

Uvo administration had a minimal impact on gut microbial diversity. Taxonomic analysis indicated relative enrichment of *Duncaniella spp.* and *Anaerotignum propionicum*. *Duncaniella spp.* belong to the *Muribaculaceae* family [28]. This family is associated with propionate production and anti-inflammatory properties [29–31]. Similarly, *Anaerotignum propionicum* has been reported to contribute to propionate synthesis [32]. Additionally, a trend toward increased relative abundance of *Akkermansia muciniphila* [33] and *Ruminococcus* spp. [34,35], both associated with short-chain fatty acid production, was noted following Uvo administration.

In contrast, Cip administration was associated with marked alterations in gut microbiota composition, accompanied by reductions in richness and evenness. It was also characterized by an increased relative abundance of *Phocaeicola vulgatus* and *Akkermansia muciniphila*. These are mucin-degrading bacteria that have been linked to intestinal mucosal homeostasis, metabolic regulation, and immune modulation [36–40]. Altered abundance of *Phocaeicola vulgatus* has been implicated in conditions such as ulcerative colitis and Crohn's disease [41–43]. For *Akkermansia muciniphila*, although beneficial effects have been documented in many contexts, its expansion under dysbiotic conditions has also been associated with excessive mucin degradation and microbial imbalance [44].

However, this study did not include a functional analysis of these microbial changes, and thus, it remains unclear whether they

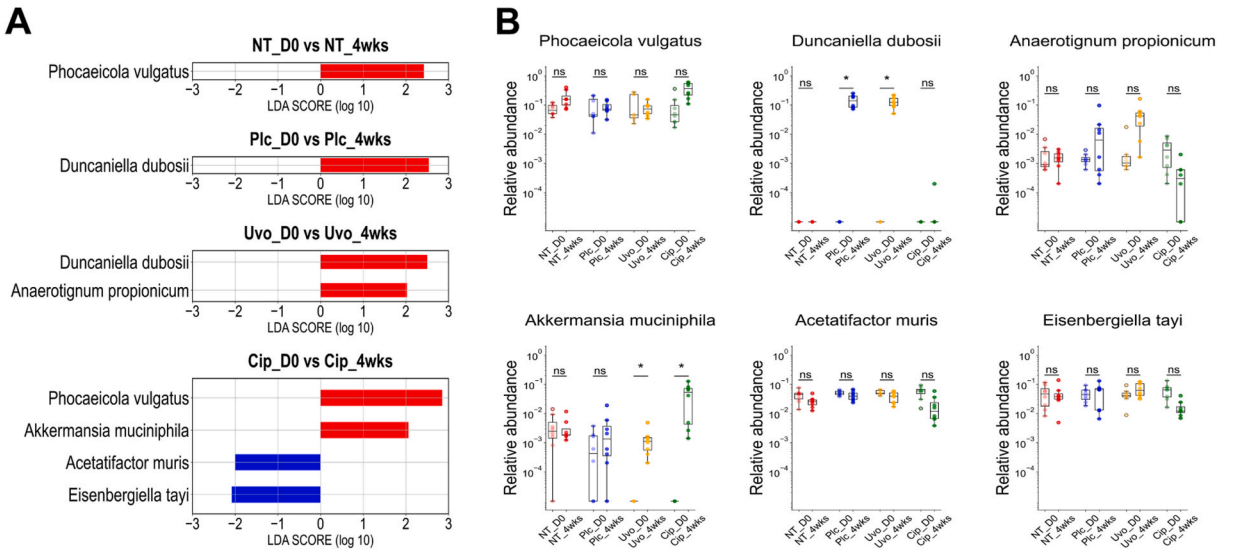

Fig. 7. Effect of drug treatment on gut microbial profiles. (A) Identification of gut microbial species following the administration of four different drugs, as determined by LEfSe analysis. (B) Relative abundances of microbial species identified through LEfSe analysis are presented by group in box plots. Each box plot represents the distribution of relative abundance within each group (n = 8 per group), indicating the median, quartiles, and outliers. Differences between groups were analyzed using a Wilcoxon signed rank test, with *p*-values adjusted using the FDR method of Benjamini and Hochberg. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

exerted beneficial or detrimental effects. Accordingly, further research is needed to clarify the functional impact of Uvo-induced alterations in the gut microbial community. Nevertheless, the overall patterns in the Uvo group were comparable to those in the Plc group, with only minor effects on richness, evenness, and dominance alongside changes in specific taxa, suggesting that microbial diversity was relatively better preserved compared with the Cip group.

Interestingly, approximately half of the species that increased or decreased following Uvo administration also showed similar trends in the Plc group, suggesting that the major components of Uvo may not have exerted a comprehensive influence on gut microbiota diversity but rather a partial effect. Instead, these findings imply that the observed microbial shifts may have been mediated by interactions with other constituents within the formulation, such as excipients present in Uvo. Pharmaceutical excipients, which typically constitute more than 90% of a drug formulation [45], are known to interact with gut microbiota, potentially altering pharmacokinetic performance and influencing microbial diversity positively or negatively. For instance, emulsifiers and surfactants (e.g., Polysorbate/Tween 80, carboxymethylcellulose) have been reported to reduce microbial diversity, and contribute to dysbiosis. In contrast, polysaccharide-based excipients (e.g., starch, gums, alginates) can serve as fermentable substrates for gut microbes, promoting microbial richness [46,47]. Uvo contains Magnesium Silicate, Magnesium Stearate, Mannitol, and Corn Starch. Mannitol and Corn Starch can serve as energy sources for certain gut microbes, potentially influencing microbial composition and diversity. This raises the possibility that the microbial changes observed in this study were influenced not only by Uvo's active components but also by its excipient composition. Given these findings, it is essential to consider excipient-microbiota interactions when evaluating drug-induced microbial changes. A deeper understanding of these interactions will be critical for optimizing pharmaceutical formulations to enhance therapeutic outcomes while minimizing unintended microbiome-related side effects.

This study has limitations in that it focused exclusively on the effects of Uvo on the gut microbiota in healthy mouse models. While the findings suggest that Uvo may have a positive impact on gut microbial diversity, the results are restricted to a healthy mouse model, making it uncertain whether similar effect would occur in rUTIs patients taking Uvo. Furthermore, current understanding remains limited regarding whether Uvo-induced changes in microbial diversity are associated with both the regulation of the gut-bladder axis and the mechanism by which rUTIs recurrence is suppressed. Another limitation is the choice of ciprofloxacin as a comparator. Although it served as a useful reference antibiotic in this study, ciprofloxacin is not generally recommended as a first-line agent for long-term rUTI prophylaxis in clinical practice, and the dosing schedule in the model does not fully reflect standard clinical regimens. Thus, while informative, the ciprofloxacin findings should be interpreted with caution, as the primary objective was to evaluate Uvo.

In addition, due to differences in the gut microbiota composition between mice and humans, further research is necessary to determine whether the effects of Uvo observed in this study would be replicated in humans. While the gut microbiota of both species exhibit high functional similarity, their taxonomic composition differs significantly, with only 4% of bacterial species being shared [48,49]. When human and mouse strains were classified as “shared species,” the functionally closest mouse strain matched the same species in 99% of cases. However, despite the fact that human *Phocaeicola dorei* appears as the same species in the mouse gut microbiota, it was functionally closer to mouse *Phocaeicola vulgatus*. Furthermore, functional mismatches became more frequent among the closest mouse species identified at higher taxonomic ranks (e.g., family or order). This indicates that the taxonomically closest neighbor is not necessarily the functionally closest species [50]. Moreover, the ratio of Firmicutes to Bacteroidetes (F/B)

notably differs between humans and mice, with humans exhibiting a higher F/B ratio [51]. The taxonomic composition also varies, with Bacteroidetes in mice primarily *Muribaculaceae* family, whereas in humans, they are predominantly represented by *Ruminococcaceae*, *Bacteroidaceae*, and *Prevotellaceae* [51,52]. Germ-free mice that were colonized with human microbiota did not achieve proper immune maturation. In contrast, those colonized with mouse microbiota demonstrated a fully developed immune response [53]. This highlights the significant differences in host-microbiota interactions between mice and humans, despite functional similarities in their gut microbial communities. Therefore, these differences underscore the limitations of directly extrapolating findings from mouse models to humans and emphasize the need for further research to elucidate the effects of Uvo on the human gut microbiota.

5. Conclusions

This study demonstrates that Uvo preserves gut microbial diversity more effectively than ciprofloxacin, indicating that it induces substantially less perturbation of the microbiota. Although Uvo is used clinically for the prevention of rUTIs, the present findings are limited to a healthy mouse model and should not be interpreted as direct evidence of clinical efficacy or underlying mechanism. Future studies using mouse models infected with uropathogenic bacteria will be necessary to determine whether the gut microbiota-preserving effects of Uvo are associated with reduced susceptibility to rUTIs. In addition, clinical studies, particularly those involving patients, will be essential to determine whether the preservation of microbial diversity observed here translates into clinical benefit. More broadly, these results underscore the importance of considering microbiota preservation when evaluating therapeutic interventions.

CRediT authorship contribution statement

Jungchan Jung: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sodam Won:** Validation, Methodology, Investigation. **Hyunho Han:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Ethics statement

All animal experiments were approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine (IACUC number: 2022 – 0106; approved by Sangwook Park). The experiments were conducted in accordance with the ARRIVE guidelines 2.0, and the institutional, national, and international regulations for the care and use of laboratory animals. Every effort was made to minimize animal suffering and to reduce the number of animals used. All authors had access to the study data and approved the final manuscript.

Data availability statement

Data will be made available on request. For requesting data, please write to the corresponding author.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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