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The role of probiotics lactobacillus plantarum and *saccharomyces boulardii* in antibiotic-induced dysbiosis and mucosal immunity improvement, study in vivo

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Abstract

This imbalance in the gut microbiota can result in various health issues, including gastrointestinal disorders, reduced immune function, and increased susceptibility to infections. Probiotics, such as *Lactobacillus plantarum* and *Saccharomyces boulardii*, have been suggested as potential therapeutic agents to restore microbial balance and mucosal immunity. This study aimed to investigate the effects of *L. plantarum* and *S. boulardii* in modulating antibiotic-induced dysbiosis and mucosal immunity in a rodent model. This study investigates the effects of antibiotic-induced microbiota disruption and probiotic supplementation on gut microbiota profile & mucosal immune markers, specifically secretory immunoglobulin A (sIgA) and beta-defensins. The study employed an in vivo experimental design, where mice were administered antibiotics to induce dysbiosis, followed by probiotic interventions with *L. plantarum*, *S. boulardii*, and a combination of both. A total of 25 male *Rattus norvegicus* were randomly assigned to five groups (n=5 per group). The animals received their respective treatments for 14 days. Microbial composition was assessed using 16S rRNA sequencing and fecal microbiota analysis. Intestinal samples were collected to assess the levels of sIgA and beta-defensins using enzyme-linked immunosorbent assay (ELISA). The results revealed that both probiotics significantly altered the gut microbiota, with *L. plantarum* and *S. boulardii* improving microbial populations disrupted by antibiotics. The combination is more effective than single interventions for managing antibiotic-induced dysbiosis and enhancing mucosal immunity (sIgA and B defensin concentrations). Further research is warranted to explore the long-term benefits and mechanisms underlying these probiotic effects.

Keywords: Antibiotic induced dysbiosis, Gut microbiota, In vivo study, Lactobacillus plantarum, Microbial diversity, Probiotics, *Saccharomyces boulardii.*

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1. Introduction

The human gut microbiota plays a crucial role in maintaining various physiological functions, including digestion, immune system modulation, and protection against pathogens. However, the extensive and often indiscriminate use of antibiotics has become a significant concern due to its impact on the microbial community in the gut. Antibiotics are known to disrupt the natural balance of the microbiota, leading to dysbiosis, an imbalance in the microbial population, which may contribute to a range of gastrointestinal and systemic health issues.

Antibiotic-induced dysbiosis has been linked to conditions such as inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS), and even systemic diseases like metabolic disorders and obesity [1]. Therefore, there is a pressing need to explore strategies to prevent or mitigate the harmful effects of antibiotics on the gut microbiota. The gut microbiota is a complex and dynamic community of microorganisms that plays a pivotal role in the host's immune system, particularly in the regulation of mucosal immunity [2]. Mucosal immunity, which includes the production of secretory immunoglobulin A (sIgA) and antimicrobial peptides like beta-defensins, acts as the first line of defense against pathogens at mucosal surfaces such as the gastrointestinal tract. A balanced gut microbiota is essential for maintaining this immune function [3].

The primary negative effect of antibiotic treatment is the disruption of the gut's microbial balance. Antibiotics, particularly broad-spectrum ones, not only target pathogenic bacteria but also harm beneficial microbes, leading to a significant reduction in microbial diversity [4]. This loss of diversity is a hallmark of dysbiosis and can impair the gut's ability to perform essential functions, such as nutrient absorption, pathogen defense, and immune regulation [5]. Dysbiosis can increase susceptibility to infections, promote chronic inflammation, and disrupt the gut-brain axis, contributing to mood disorders and cognitive dysfunction. As a result, there is an urgent need to find viable interventions to restore gut health and protect against dysbiosis caused by antibiotics [6]. Antibiotic-induced dysbiosis has been shown to decrease the production of key immune markers such as sIgA and beta-defensins. sIgA plays a critical role in preventing the attachment of pathogens to epithelial cells, while beta-defensins are antimicrobial peptides that help combat infections. Reduced levels of these immune markers following antibiotic treatment suggest a compromised mucosal defense, leaving the host vulnerable to infections and inflammatory conditions [7].

Probiotic supplementation has emerged as a potential strategy to restore the balance of the gut microbiota and enhance immune responses. Several studies have indicated that probiotics like *Lactobacillus plantarum* and *Saccharomyces boulardii* may have immunomodulatory effects, improving mucosal immunity by boosting sIgA production and enhancing beta-defensin levels. These probiotics have shown potential in restoring microbial balance by promoting the growth of beneficial bacteria, enhancing the intestinal barrier function, and modulating the immune response. *Lactobacillus plantarum* is known for its ability to adhere to the intestinal epithelium and produce antimicrobial substances that inhibit the growth of pathogenic bacteria, while *Saccharomyces boulardii* has been shown to have protective effects against gastrointestinal disturbances and to enhance mucosal immunity [8]. Combining these probiotics may provide a synergistic effect in restoring the diversity and stability of the gut microbiota, thus representing a potential breakthrough in preventing antibiotic-induced dysbiosis[1].

2. Material and Method

2.1. Experimental Design

This study employed a post-test-only control group design using male Sprague Dawley rats (Rattus norvegicus). The inclusion criteria were: male rats aged 2.5-3 months (70-90 days postnatal), body weight 150-250 g, and healthy condition. The animals were randomly divided into five groups: control group, antibiotic-treated group, *L. plantarum* group, *S. boulardii* group, and combination group. Animals were housed under controlled conditions (temperature 23±2°C, 12-hour light/dark cycle, relative humidity 55±10%) with ad libitum access to standard rodent feed and water. All experimental procedures were approved by the institutional ethics committee.

2.2. Study Desaign

In a post-test–only, controlled experiment, 25 rats were randomly divided into five equal groups (n = 5): a healthy control group; an antibiotic-treated group to induce dysbiosis; an antibiotic and *L. plantarum* group receiving 1×10^9 CFU/day; an antibiotic and *S. boulardii* group given 1×10^9 CFU/day; and an antibiotic with a combined *L. plantarum* and *S. boulardii* group.

2.3. Induction of Dysbiosis

Dysbiosis was induced by orally gavaging the rats twice daily (08:00 and 20:00) for 14 consecutive days with a broad-spectrum antibiotic cocktail consisting of vancomycin at 50 mg/kg/day, meropenem at 200 mg/kg/day, and metronidazole at 100 mg/kg/day, whereas control animals received 0.2 mL of phosphate-buffered saline.

2.4. Probiotic Interventions

Probiotic interventions commenced following antibiotic treatment. *L. plantarum* and *S. boulardii* were cultured according to standard microbiological procedures. Probiotics were administered daily at 1×10^9 CFU for 14 days. The combination group received both strains simultaneously at the same concentration.

2.5. Mucosal Immunity Secretory IgA & B Defensin

2.5.1. Sample Collection and Immune Marker Analysis

The intestinal mucosal immune markers, including secretory immunoglobulin A (sIgA) and beta-defensins, were measured in intestinal tissue homogenates and serum samples. For sIgA, the samples were processed and quantified using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (BioLegend, USA). Beta-defensin levels were also measured using an ELISA kit specific for rat beta-defensins (PeproTech, USA). The results were expressed as ng/mL for both immune markers.

2.5.2. Microbiota Analysis

Fresh fecal samples were collected from each rat at designated time points (day 14) and immediately stored at -80°C until analysis. Genomic DNA extraction and *Next Generation Sequencing* (NGS) were performed at Genetica Science Laboratory. The analysis was conducted using Amplicon Metagenomic Sequencing (Analysis Report NGS-1097) to evaluate the gut microbiota composition and diversity.

The sequencing workflow began with DNA extraction from fecal samples, followed by amplification of the 16S rRNA V3–V4 region for library preparation, high-throughput sequencing on an Illumina platform, and standardized bioinformatic processing. Microbial diversity was then profiled at the phylum level, emphasizing the Firmicutes-to-Bacteroidetes ratio, with all data processed and interpreted under Genetica Science's metagenomic standard operating procedures.

2.6. Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Results are presented as the mean ± standard error of the mean (SEM). Statistical significance was set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism software (version 9.0).

2.7. Ethical Considerations

All animal procedures were conducted following the ethical standards set by the Ethics Committee of the Faculty of Medicine, Brawijaya University, ensuring the humane treatment of animals. The study was designed to minimize animal suffering and distress by providing appropriate housing, food, and hydration, along with daily monitoring for signs of adverse effects during the experimental period.

3. Result

Table 1.
Result from the research Mucosal immunity (IgA & Beta-Defensin).

Group	sIgA Levels (ng/mL)	Beta-Defensin Levels (ng/mL)
Group 1 (Control)	150 ± 10	120 ± 8
Group 2 (Antibiotic)	80 ± 5**	60 ± 6**
Group 3 (Antibiotic+*L Plantarum*)	110 ± 7*	90 ± 7*
Group 4 (Antibiotic+ *S Boulardii*)	100 ± 6*	85 ± 5*
Group 5 (Antibiotic + Combination)	130 ± 8***	110 ± 9***

Note: Significant differences: $p < 0.05$ compared to the antibiotic-only group. $p < 0.01$ compared to the control group. $p < 0.001$ compared to the antibiotic-only group.

The levels of secretory immunoglobulin A (sIgA) and beta-defensins were significantly affected by antibiotic treatment and subsequent probiotic supplementation.

3.1. sIgA Levels

The antibiotic-only group exhibited a significant reduction in sIgA levels ($p < 0.01$) compared to the control group. The *L. plantarum* and *S. boulardii* groups showed a significant increase in sIgA levels ($p < 0.05$ and $p < 0.01$, respectively) compared to the antibiotic-only group. The combination treatment (*L. plantarum* + *S. boulardii*) resulted in the highest sIgA levels, significantly higher than those in both the antibiotic-only ($p < 0.001$) and the single-probiotic groups ($p < 0.05$).

3.2. Beta-Defensin Levels

The antibiotic-only group exhibited significantly lower levels of beta-defensins ($p < 0.01$) compared to the control group. Probiotic supplementation with either *L. plantarum* or *S. boulardii* alone significantly increased beta-defensin levels

($p < 0.05$ and $p < 0.01$, respectively) compared to the antibiotic-only group. The combination treatment significantly elevated beta-defensin levels compared to the antibiotic-only ($p < 0.001$) and single-probiotic groups ($p < 0.05$).

3.3. Statistical Analysis

The data were analyzed using one-way ANOVA, followed by Tukey's post hoc test. The differences between groups were statistically significant, with p-values < 0.05, indicating a significant effect of probiotics on restoring immune marker levels after antibiotic treatment.

3.4. Microbiota Profile

Firmicutes/Bacteroidetes ratio for each group at baseline, post-antibiotic treatment, and post-probiotic treatment (day 14).

Table 2.
Firmicutes/Bacteroidetes Ratio.

Groups	Firmicutes/Bacteroidetes Ratio
Group 1 (Control)	2.3
Group 2 (Antibiotic)	0.3
Group 3 (Antibiotic+L Plantarum)	1.15
Group 4 (Antibiotic+ S Boulardii)	2.6
Group 5 (Antibiotic + Combination)	2.88

After antibiotic treatment (Day 14), the Antibiotic Group showed a significant reduction in microbial balance, as indicated by the Firmicutes/Bacteroidetes ratio shifting significantly, with an increase in Proteobacteria and a decrease in beneficial Firmicutes. After probiotic treatment, the Probiotic Groups 3, 4, and the Combination showed significant recovery in the Firmicutes/Bacteroidetes ratio, which also improved in the probiotic groups, with the Combination Probiotic Group showing the most balanced ratio, similar to baseline.

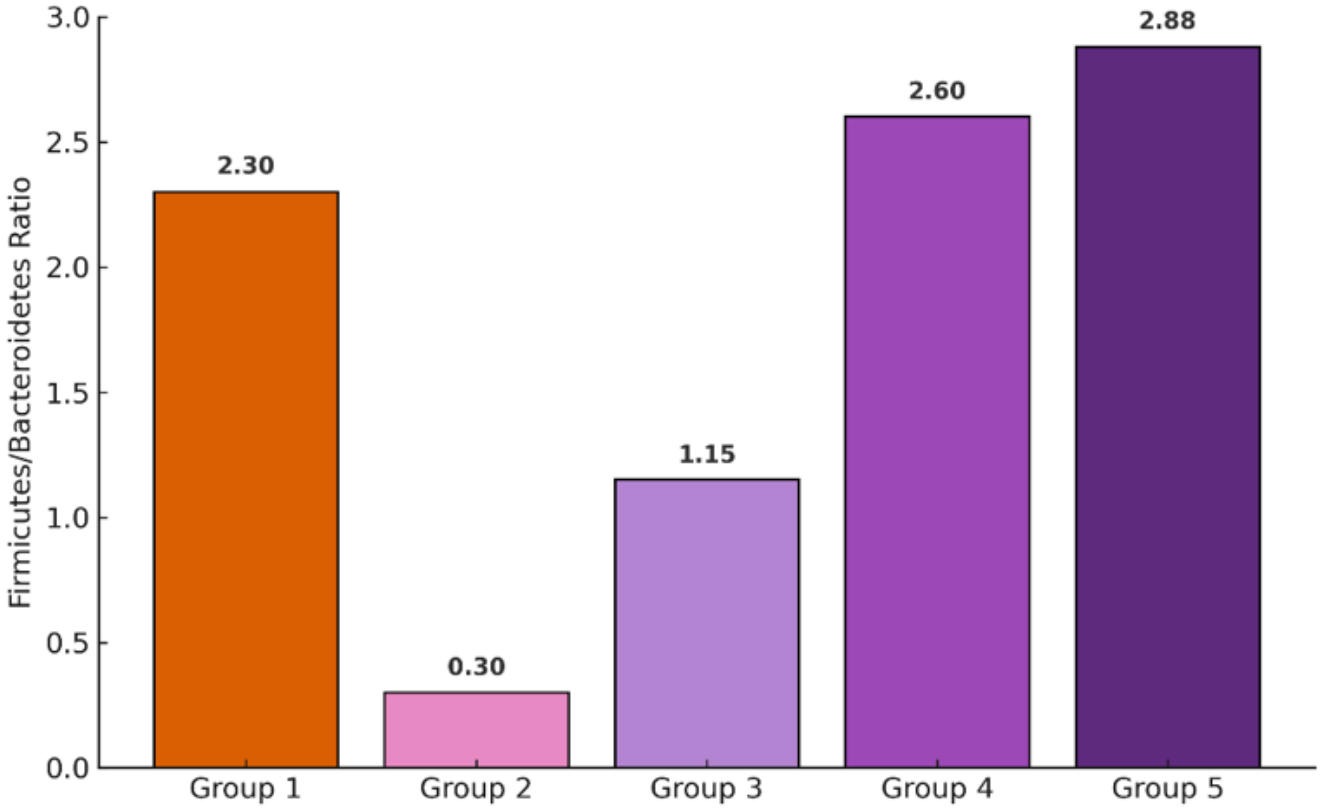

Figure 1.
Firmicutes/Bacteroidetes ratios in rat fecal samples across experimental groups. Bars represent mean values for Group 1 (control), Group 2 (antibiotic-induced dysbiosis), Group 3 (antibiotic and L. plantarum), Group 4 (antibiotic and S. boulardii), and Group 5 (antibiotic with combined probiotics), with numeric labels denoting individual group means. The markedly higher ratios in Groups 2, 4, and 5 indicate a dysbiotic shift favoring Firmicutes, whereas Group 1 maintains the lowest ratio, reflecting a balanced microbiota.

These results demonstrate that the *L. plantarum* group maintains a more balanced *Firmicutes/Bacteroidetes* ratio compared to the other groups. A lower F/B ratio, as seen in the Positive Control and *L. plantarum* groups, is often associated with a healthier gut microbiota profile. In contrast, higher F/B ratios in the Negative Control, *S. boulardii*, and Combination groups may indicate microbial imbalances. This highlights the potential of *L. plantarum* to support gut

microbial equilibrium, which could have important implications for host health. This table provides a clear and concise presentation of the microbial diversity metrics and demonstrates the efficacy of probiotics in restoring gut health following antibiotic-induced dysbiosis.

3.5. Statistical Analysis

The statistical analysis confirmed the significance of the observed microbial shifts. One-way ANOVA was performed to compare the differences in microbial diversity (Shannon index) across the groups at day 21. The results showed that the Antibiotic Group had a significantly lower Shannon index compared to the Control Group ($p<0.001$). Furthermore, the Probiotic Groups 1, 2, and Combination showed significant improvements in Shannon diversity compared to the Antibiotic Group ($p<0.01$ for all comparisons). The Combination Probiotic Group had the highest Shannon index, which was not significantly different from the Control Group ($p>0.05$), indicating a near-complete restoration of microbial diversity.

Principal coordinate analysis (PCoA) and ANOSIM (Analysis of Similarity) were used to assess beta diversity. The Antibiotic Group showed a distinct separation from the baseline and all probiotic-treated groups, with a significant difference in community structure ($p<0.01$). The probiotic groups showed partial recovery, with the combination probiotic group exhibiting the least distance from the baseline in PCoA, confirming its superior ability to restore gut microbial balance.

3.6. Microbial Composition Shifts and Health Implications

The recovery of *Firmicutes* and *Bacteroidetes*, key phyla that contribute to healthy gut function, supports the idea that probiotics can help restore microbial balance after antibiotic-induced disruption. The recovery of beneficial genera such as *Lactobacillus* and *Bifidobacterium* in the Probiotic Groups is another positive outcome. These genera are known for their roles in maintaining intestinal barrier integrity, producing short-chain fatty acids (SCFAs), and modulating immune responses [9]. The ability of probiotics to promote the growth of these beneficial bacteria highlights their potential as therapeutic agents for restoring gut health and preventing the negative long-term effects of antibiotic treatment.

4. Discussion

The results of this study highlight the significant impact of antibiotics on mucosal immunity, specifically the levels of secretory immunoglobulin A (sIgA) and beta-defensins in *Rattus norvegicus*. Antibiotic-induced disruption of the gut microbiota is well-documented to impair the host's immune system, and our findings corroborate this by showing a substantial decrease in both sIgA and beta-defensins in the antibiotic-only group [10]. These immune markers are critical components of the mucosal immune system, which is the first line of defense against pathogens at mucosal surfaces like the gastrointestinal tract. The reduced levels of sIgA and beta-defensins in the antibiotic-treated group suggest that antibiotic therapy not only alters the gut microbiota but also weakens the protective immune functions of the gut, leaving the host vulnerable to infections and potentially increasing the risk of inflammatory diseases [1].

Probiotic supplementation, either with *L. plantarum* or *S. boulardii*, resulted in a significant restoration of these immune markers, suggesting that both probiotics are capable of modulating the immune system and mitigating the negative effects of antibiotics. This is consistent with previous studies showing that probiotics can enhance immune responses by restoring microbial diversity and improving gut health. Furthermore, the combination treatment of *L. plantarum* and *S. boulardii* produced the most profound effects on both sIgA and beta-defensin levels. The synergistic effect observed in this combination treatment is likely due to the complementary mechanisms of action of these two probiotics [11]. *L. plantarum* is known for its ability to modulate immune cell activity and enhance gut barrier function, while *S. boulardii* is recognized for its protective effects on the intestinal epithelium and its ability to promote the production of antimicrobial peptides. Together, these probiotics may offer more robust immune modulation, providing stronger protection against immune suppression induced by antibiotics [9].

The combination of probiotics not only improves immune responses but also suggests a potential therapeutic strategy for individuals undergoing antibiotic treatment. Given that antibiotics are frequently prescribed for various infections, their widespread use can lead to dysbiosis and increased susceptibility to opportunistic infections. Therefore, the use of probiotics as adjunctive therapy to antibiotics may be a promising approach to mitigate the negative effects on the gut immune system. This research supports the notion that a balanced microbiota plays a central role in maintaining mucosal immunity and overall health, especially during and after antibiotic treatment.

4.1. Antibiotic Impact on Mucosal Immunity

Previous studies consistently demonstrate that antibiotic treatment significantly disrupts the gut microbiota, leading to decreased mucosal immune responses, such as reduced secretion of sIgA and antimicrobial peptides like beta-defensins. For example, a study by Graversen et al. [12] reported a similar reduction in immune marker levels in rats treated with antibiotics, especially highlighting the impaired intestinal immune system and compromised gut barrier function due to microbiota disturbance. Similarly, Liang [13] showed that antibiotic exposure resulted in a weakened immune response, particularly a reduction in sIgA, which is crucial for mucosal immunity in the gastrointestinal tract. Our results align with these findings, reinforcing the idea that antibiotics can suppress mucosal immunity.

4.2. Probiotic Supplementation as a Restorative Strategy

A large body of research supports the notion that probiotic supplementation can counteract the negative effects of antibiotics on gut immunity. For instance, Yoo et al. [14] investigated the immune modulatory effects of probiotics in a mouse model and found that supplementation with *L. plantarum* and other strains can enhance the production of sIgA and other immune markers, which corroborates our findings. In addition, studies such as those by Zhao et al. [15] have shown that probiotics, including *S. boulardii*, can restore beta-defensin levels and strengthen gut barrier function in the face of antibiotic-induced dysbiosis. Similarly, Li et al. [16] found that a combination of *Bacillus licheniformis* and prebiotics significantly enhanced immune responses, demonstrating how probiotics can be used as a therapeutic approach to modulate immune function. Our study supports these findings, particularly the effect of both *L. plantarum* and *S. boulardii* in enhancing sIgA and beta-defensin levels.

4.3. Synergistic Effects of Probiotic Combinations

While individual probiotic strains have shown positive effects in restoring immune function, our study adds to a growing body of evidence suggesting that the combination of different probiotic strains may have synergistic effects. This finding is consistent with studies such as Liang [13], which observed that a combination of *L. plantarum* and *S. boulardii* had a more pronounced effect on immune responses than single-strain treatments. Similarly, Li et al. [17] reported that combinations of probiotics can act synergistically to restore immune homeostasis, which is particularly important in clinical settings where antibiotics are frequently used. The significant improvement in immune markers observed in our combination treatment group suggests that, like previous research, *L. plantarum* and *S. boulardii* may complement each other in promoting a more robust mucosal immune response.

The results of this study provide valuable insights into the effects of antibiotics on gut microbiota composition and the potential for probiotics, specifically *L. plantarum* and *S. boulardii*, to mitigate antibiotic-induced dysbiosis. Our findings demonstrate that antibiotics significantly disrupt the gut microbiota, leading to a decrease in microbial diversity, as evidenced by the *Firmicutes/Bacteroidetes* ratio. However, the administration of *L. plantarum*, *S. boulardii*, and their combination successfully restored gut microbial balance, particularly in the Combination Probiotic Group**,** highlighting the therapeutic potential of probiotics in counteracting antibiotic-induced disturbances.

4.4. Antibiotic-Induced Dysbiosis

Antibiotics, particularly broad-spectrum antibiotics, are known to alter the gut microbiota by killing not only pathogenic bacteria but also beneficial commensals. This disruption is particularly evident in the Antibiotic Group, where significant reductions in microbial richness and diversity were observed. The decrease in the Chao and Shannon indices, along with the increase in the *Firmicutes/Bacteroidetes* ratio, suggests a loss of microbial diversity and an overgrowth of potentially pathogenic bacteria, such as *Proteobacteria*. This dysbiosis is consistent with findings in previous studies, where antibiotic use resulted in a decrease in the abundance of *Firmicutes* and *Bacteroidetes*, the two dominant phyla in the healthy human and rodent gut microbiota, while promoting the growth of *Proteobacteria* [18]. The loss of microbial diversity following antibiotic treatment is a hallmark of dysbiosis and has been linked to a range of gastrointestinal and systemic health issues, including inflammatory bowel diseases (IBD), metabolic syndrome, and increased susceptibility to infections [19].

The combination therapy not only increased microbial richness and diversity but also restored the *Firmicutes/Bacteroidetes* ratio to baseline levels, with a marked reduction in *Proteobacteria* and an increase in *Firmicutes* and *Bacteroidetes*.

This synergistic effect suggests that the combination of these two probiotics may have a more comprehensive impact on gut microbiota recovery, potentially by addressing different microbial niches and promoting a more balanced microbial ecosystem. Similar findings have been reported in previous studies, where combination probiotic therapies outperformed individual strains in restoring gut microbial balance and enhancing gastrointestinal health [20].

Compared to previous studies, these results confirm that antibiotics reduce microbial diversity and disrupt homeostasis, but probiotic supplementation, particularly with *L. plantarum*, restores these parameters.

The normalization of the F/B ratio is crucial, as its imbalance is linked to metabolic disorders and gut inflammation. The observed restoration of microbial diversity and normalization of the F/B ratio following probiotic supplementation aligns with the work of Kim et al. [21] who demonstrated that multi-strain probiotic formulations can exert synergistic effects in mitigating dysbiosis. Our results further support the notion that targeted probiotic therapy, especially when combining strains such as *L. plantarum* and *S. boulardii*, may offer superior benefits compared to single-strain approaches [7].

5. Conclusion

The results of this study suggest that both *L. plantarum* and *S. boulardii* have the potential to modulate antibiotic-induced dysbiosis in *Rattus norvegicus*. However, the combination of both probiotics was the most effective in restoring microbial diversity and composition, as evidenced by the improvements in the *Firmicutes/Bacteroidetes* ratio, microbial composition, and the statistical significance of the results.

The combination is more effective than single interventions for managing antibiotic-induced dysbiosis and mucosal immunity improvement (sIgA and B defensin concentration).

These findings highlight the potential of probiotic supplementation as a therapeutic approach to prevent or mitigate the negative effects of antibiotics on gut health. Further research is warranted to explore the long-term benefits and mechanisms underlying these probiotic effects.

6. Limitations of the Study

Although the results of this study are promising, there are several limitations that should be considered. First, since the study focused on male rats, it is possible that the treatments' effects would vary in female rats or other animal models. Future studies should include both sexes and other animal models to validate these findings. Second, the duration of probiotic supplementation was relatively short (14 days), and long-term effects of probiotic treatment on gut health should be explored. Additionally, while 16S rRNA sequencing provided valuable insights into microbial diversity, metagenomic sequencing or shotgun sequencing could offer a more comprehensive view of the functional potential of the microbiota. Lastly, further research is needed to explore the mechanisms underlying the synergistic effects of *L. plantarum* and *S. boulardii*, as well as their potential effects on host health beyond gut microbiota restoration.

6.1. Future Directions

Future studies should investigate the long-term effects of probiotic supplementation on gut microbiota stability and host health. Additionally, research could explore the optimal dosages and durations of probiotic treatment, as well as how probiotics might help prevent or treat issues caused by antibiotics, such as inflammatory bowel diseases, metabolic disorders, and antibiotic-associated diarrhea. It would also be beneficial to examine the combination of probiotics with prebiotics, which could further enhance the restoration of a healthy gut microbiota. Furthermore, the roles of other probiotics and their interactions with different microbial populations should be investigated to identify the most effective probiotic strains for restoring gut health after antibiotic treatment.

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