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Prevention of Normal Escherichia Coli Flora Resistance by Using Probiotics in Combination Antibiotic-Induced Mice

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ABSTRACT

The indiscriminate use of broad-spectrum antibiotics can cause gut microbiota dysbiosis and select for resistant bacteria, including commensal Escherichia coli. Probiotics such as Lactobacillus plantarum and Saccharomyces boulardii are believed to restore microbiota balance and prevent antibiotic resistance. This research aimed to evaluate the effectiveness of L. plantarum, S. boulardii, and their combination in preventing antibiotic resistance in commensal E. coli in the intestines of Wistar rats induced with a combination of antibiotics. An experimental post-test control group design was conducted using 25 male Wistar rats divided into five groups: negative control, positive control (antibiotics only), and three treatment groups (antibiotics + L. plantarum, antibiotics + S. boulardii, antibiotics + both). The resistance profile of E. coli from fecal samples was analyzed on days 7 and 14 using the Kirby-Bauer method against ceftazidime, meropenem, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. Statistical analysis used Independent Sample T-Test ($p < 0.05$). The positive control group showed a significant reduction in antibiotic inhibition zones, indicating increased E. coli resistance. In the L. plantarum (alone or combination) groups, no E. coli growth was found in fecal cultures. The S. boulardii group showed significantly higher antibiotic sensitivity compared to the positive control ($p < 0.05$). Conclusion: Administration of probiotics, particularly L. plantarum and its combination with S. boulardii, was effective in preventing antibiotic resistance in commensal E. coli after induction with broad-spectrum antibiotics in rats. These findings support the use of probiotics as adjuncts to antibiotic therapy to suppress resistance development at the gut microbiota level.

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INTRODUCTION

Antibiotic resistance is a critical global health threat, driven by the overuse and misuse of antibiotics in clinical and agricultural settings (Aljeldah, 2022; Berndtson, 2020). The World Health Organization projects millions of deaths annually by 2050 due to untreatable infections if resistance is not controlled (Alara & Alara, 2024; Coque et al., 2023; Nazir et al., 2025). Broad-spectrum antibiotics disrupt gut microbiota, resulting in dysbiosis and selection of resistant strains (Chen et al., 2021). *Escherichia coli*, a dominant commensal in the gut, can act as a reservoir for resistance genes and become an opportunistic pathogen, especially after antibiotic exposure (Aljeldah, 2022). The emergence of multidrug-resistant *E. coli* strains, particularly those resistant to third-generation cephalosporins and carbapenems, is classified as a critical priority by WHO (Hayer et al., 2022; Puljko, 2024; Ruef et al., 2024; White, 2021).

In Indonesia, the Ministry of Health surveillance data indicate that *E* (Hardhantyo et al., 2022; Mboi et al., 2022). *coli* resistance rates to third-generation cephalosporins have reached 60–80% in major hospitals, while carbapenem resistance has increased to 15–25% over the past five years (Mark et al., 2021; Moghnieh et al., 2021; Silva, 2022). WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) data show that Indonesia ranks among the top countries for multidrug-resistant *E. coli* prevalence in Southeast Asia, with particularly high resistance rates in intensive care units and neonatal wards (Dickson et al., 2025).

Previous research has established the individual efficacy of probiotic strains in preventing antibiotic-associated complications. Hedin (2022) demonstrated that *Lactiplantibacillus plantarum* administration reduced *E. coli* colonization by 75% in antibiotic-treated mice, while maintaining microbiota diversity. Similarly, Sharif (2023) showed that *Saccharomyces boulardii* supplementation restored gut barrier function and reduced pathogen translocation in antibiotic-induced dysbiosis models. Liao (2025) reported that *S. boulardii* produced metabolites that directly inhibited *E. coli* biofilm formation and reduced horizontal gene transfer of resistance genes. However, comparative studies analyzing the relative effectiveness of *L. plantarum* versus *S. boulardii* have yielded mixed results, with *L. plantarum* showing superior bacteriocin production, but *S. boulardii* demonstrating better immune modulation properties.

Critical knowledge gaps remain regarding the optimal probiotic combinations and dosing strategies for preventing antibiotic resistance (Elshaghabee & Rokana, 2022; Zavišić et al., 2023). Most previous studies have focused on single probiotic strains, with limited investigation of synergistic effects (Han et al., 2024; McFarland, 2021). The mechanisms underlying probiotic-mediated resistance prevention are incompletely understood, particularly regarding the interaction between different probiotic species and their collective impact on gut microbiota recovery and resistance gene expression (Montassier et al., 2021; Mousa et al., 2023).

Probiotics such as *Lactiplantibacillus plantarum* and *Saccharomyces boulardii* have shown promise in restoring gut microbiota balance, inhibiting colonization by resistant bacteria, and modulating immune responses (Abid et al., 2022; Aljohani et al., 2024). Previous studies demonstrate that these probiotics can reduce colonization and transmission of resistant *E. coli* after antibiotic exposure (Hedin et al., 2022). The urgency of this research is underscored by the rapid emergence of pan-drug-resistant *E. coli* strains and the limited pipeline of new antibiotics. Current clinical guidelines lack evidence-based recommendations for probiotic use in preventing antibiotic resistance, creating an immediate need for controlled experimental data to inform therapeutic protocols.

This research is novel because it is the first to systematically compare the effectiveness of *L. plantarum* and *S. boulardii* individually versus their combination in preventing antibiotic resistance in commensal *E. coli* using a Wistar rat model with combined broad-spectrum antibiotic exposure. Unlike previous studies that focused on single probiotic strains or limited antibiotic panels, this research evaluates multi-strain synergistic effects against a clinically relevant combination of antibiotics representing different resistance mechanisms.

This study aims to evaluate the effectiveness of *L. plantarum*, *S. boulardii*, and their combination in preventing antibiotic resistance in commensal *E. coli* in Wistar rats induced with combination antibiotics. The potential benefits of this research include: (1) providing evidence-

based protocols for probiotic supplementation during antibiotic therapy, (2) informing clinical guidelines for antimicrobial stewardship programs, (3) supporting the development of standardized probiotic formulations for hospital use, and (4) contributing to strategies for reducing healthcare-associated infections and treatment costs related to antibiotic-resistant infections.

RESEARCH METHOD

This experimental study used a post-test control group design. Twenty-five healthy male Wistar rats (2–3 months, 150–200 g) were randomly assigned to five groups: negative control, positive control (combination antibiotics: meropenem, vancomycin, metronidazole), and three treatment groups (combination antibiotics + L. plantarum, antibiotics + S. boulardii, antibiotics + both). Antibiotics were administered orally twice daily for 14 days. Probiotics were administered orally once daily: L. plantarum and S. boulardii at 0.5 mL (10^9 CFU/mL) each, and the combination group received 1 mL (0.5 mL each strain).

Fecal samples were collected on days 7 and 14 for E. coli isolation on EMB agar, confirmed biochemically. Antibiotic resistance profiles were assessed using the Kirby-Bauer disk diffusion method (CLSI M100, 34th ed.) for ceftazidime/CAZ, meropenem/MEM, ciprofloxacin/CIP, gentamicin/GEN, and trimethoprim-sulfamethoxazole/SXT. Inhibition zones were interpreted according to CLSI standards. Statistical analysis used independent t-tests, with significance set at $p<0.05$.

RESULTS AND DISCUSSION

All groups were comparable in baseline characteristics. The positive control group showed significant reductions in antibiotic inhibition zones for E. coli on days 7 and 14, indicating increased resistance. In the L. plantarum and combination groups, no E. coli growth was detected in fecal cultures at either time point. The S. boulardii group showed significantly higher antibiotic sensitivity compared to the positive control ($p<0.05$).

Table 1. Mean Inhibition Zones (mm) of E. coli on Day 14

Group	CAZ	MEM	CIP	GEN	SXT
Negative Control	24,6	28,2	26,4	22,8	20,4
Positive Control	10,2	12,4	11,8	9,6	8,4
L. plantarum	-	-	-	-	-
S. boulardii	22,0	25,8	23,6	21,2	19,0
Combination	-	-	-	-	-

"-" indicates no *E. coli* growth detected.
Source: Primary data analysis, 2024

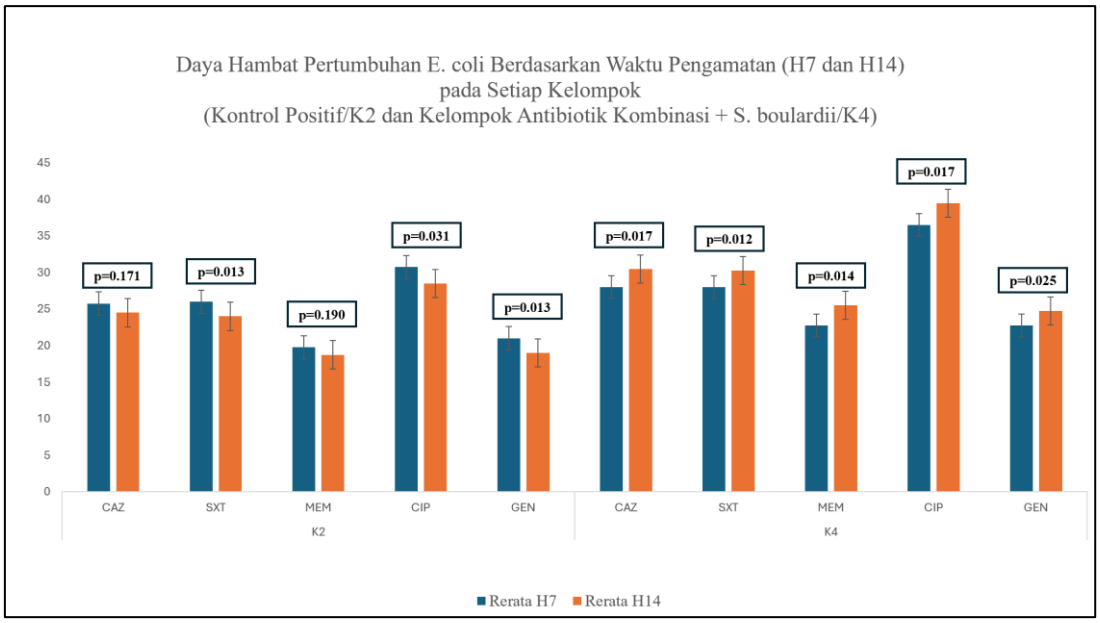

Figure 1. Comparison chart of the inhibition zones of each antibiotic between groups (positive control/K2 and S. boulardii and combination antibiotics group/K4) at each observation time point (Day 7 and Day 14).
Source: Primary data analysis, 2024

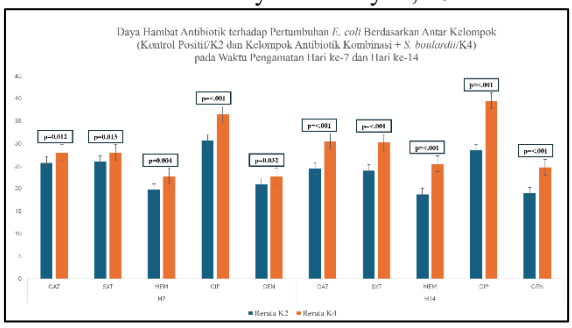

Figure 2. Comparison chart of the inhibition zones of each antibiotic at different observation times (Day 7 and Day 14) in each group (positive control/K2 and S. boulardii and combination antibiotics group/K4).

Source: Primary data analysis, 2024

This study demonstrates that combination antibiotic therapy induces significant resistance in commensal E. coli, as evidenced by reduced inhibition zones. These results are consistent with global reports that broad-spectrum antibiotics disrupt gut microbiota and select for resistant strains. Dysbiosis leads to a decrease in beneficial bacteria and a dominance of opportunistic pathogens such as E. coli, increasing the risk of resistance and secondary infections.

The absence of E. coli in the L. plantarum and combination groups suggests that these probiotics can eliminate or suppress E. coli colonization after antibiotic exposure, likely via production of organic acids, bacteriocins, competitive exclusion, and enhancement of mucosal barrier integrity Aljohani, Carvalho, Fidanza. L. plantarum is known to lower gut pH, produce plantaricin, and strengthen tight junctions, all of which inhibit E. coli growth and resistance. L. plantarum also inhibits E. coli adhesion and biofilm formation, and enhances antimicrobial peptide production. In the S. boulardii group, although E. coli was not eliminated, antibiotic sensitivity was restored, likely due to increased SCFA-producing bacteria, immune modulation, and reduced gut permeability Abid, Czerucka & Rampal, Gao. S. boulardii also enhances microbiota diversity, strengthens the gut barrier, and suppresses pro-inflammatory cytokines.

The synergistic effect in the combination group supports evidence that multi-strain probiotics can accelerate microbiota recovery and reduce pathogen colonization after antibiotic treatment. Meta-analyses show that combining L. plantarum and S. boulardii is more effective in reducing antibiotic-associated diarrhea and gut infections than single-strain probiotics. Limitations: This study used an animal model, limiting generalizability to humans. The intervention duration was short, and the antibiotic panel limited. No genotypic or molecular confirmation of phenotypic resistance was performed, so the underlying genetic mechanisms remain unclear. Further clinical studies with longer observation, broader antibiotic panels, and molecular confirmation are needed.

CONCLUSION

This research successfully achieved its objective of evaluating the effectiveness of L. plantarum, S. boulardii, and their combination in preventing antibiotic resistance in commensal E. coli in Wistar rats induced with combination antibiotics. The results demonstrate that administration of probiotics, particularly L. plantarum alone or in combination with S. boulardii, effectively prevents antibiotic resistance in commensal E. coli after induction with broad-spectrum antibiotics. These findings provide crucial evidence supporting the adjunctive use of probiotics in antibiotic therapy to suppress resistance development at the gut microbiota level. Contributions to future research include: (1) establishing a standardized animal model for evaluating probiotic interventions against antibiotic resistance, (2) providing baseline data for dose-response studies and optimization of probiotic combinations, (3) informing the design of clinical trials investigating probiotic supplementation protocols in hospitalized patients receiving broad-spectrum antibiotics, and (4) supporting the development of evidence-based guidelines for antimicrobial stewardship programs incorporating probiotic interventions. Further clinical studies are needed to confirm these effects in humans and establish optimal dosing regimens for clinical application.

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REFERENCES

Abid, R., Waseem, H., Ali, J., Ghazanfar, S., Muhammad Ali, G., Elasbali, A. M., & Alharethi, S. H. (2022). Probiotic yeast Saccharomyces: Back to nature to improve human health. *Journal of Fungi*, *8*(5), 444.

Alara, J. A., & Alara, O. R. (2024). An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis. *Infectious Disorders-Drug TargetsDisorders)*, *24*(3), 26–42.

Aljeldah, M. M. (2022). Antimicrobial resistance and its spread is a global threat. *Antibiotics*, *11*(8), 1082.

Aljohani, A., Rashwan, N., Vasani, S., Alkhawashki, A., Wu, T. T., Lu, X., Castillo, D. A., & Xiao, J. (2024). The health benefits of probiotic Lactiplantibacillus plantarum: a systematic review and meta-analysis. *Probiotics and Antimicrobial Proteins*, 1–20.

Berndtson, A. E. (2020). Increasing globalization and the movement of antimicrobial resistance between countries. *Surgical Infections*, *21*(7), 579–585.

Chen, Y., Zhou, J., & Wang, L. (2021). Role and mechanism of gut microbiota in human disease. *Frontiers in Cellular and Infection Microbiology*, *11*, 625913.

Coque, T. M., Cantón, R., Pérez-Cobas, A. E., Fernández-de-Bobadilla, M. D., & Baquero, F. (2023). Antimicrobial resistance in the global health network: known unknowns and challenges for efficient responses in the 21st century. *Microorganisms*, *11*(4), 1050.

Dickson, B. F. R., Harrison, M., Villanueva-Uy, M. E. T., Putri, N. D., Adrizain, R., Kartina, L., Gunaratna, G. P. S., Tran, H. T., Huong, N. X., & Fong, S. M. (2025). Pathogen distribution and antimicrobial resistance among neonatal bloodstream infections in

Southeast Asia: results from NeoSEAP, a multicentre retrospective study. *The Lancet Regional Health–Western Pacific*, *62*.

Elshaghabee, F. M. F., & Rokana, N. (2022). Mitigation of antibiotic resistance using probiotics, prebiotics and synbiotics. A review. *Environmental Chemistry Letters*, *20*(2), 1295–1308.

Han, D., Zulewska, J., Xiong, K., & Yang, Z. (2024). Synergy between oligosaccharides and probiotics: From metabolic properties to beneficial effects. *Critical Reviews in Food Science and Nutrition*, *64*(13), 4078–4100.

Hardhantyo, M., Djasri, H., Nursetyo, A. A., Yulianti, A., Adipradipta, B. R., Hawley, W., Mika, J., Praptiningsih, C. Y., Mangiri, A., & Prasetyowati, E. B. (2022). Quality of national disease surveillance reporting before and during COVID-19: A mixed-method study in Indonesia. *International Journal of Environmental Research and Public Health*, *19*(5), 2728.

Hayer, S. S., Casanova-Higes, A., Paladino, E., Elnekave, E., Nault, A., Johnson, T., Bender, J., Perez, A., & Alvarez, J. (2022). Global distribution of extended spectrum cephalosporin and carbapenem resistance and associated resistance markers in Escherichia coli of swine origin–a systematic review and meta-analysis. *Frontiers in Microbiology*, *13*, 853810.

Hedin, K. A., Rees, V. E., Zhang, H., Kruse, V., Vazquez-Uribe, R., & Sommer, M. O. A. (2022). Effects of broad-spectrum antibiotics on the colonisation of probiotic yeast Saccharomyces boulardii in the murine gastrointestinal tract. *Scientific Reports*, *12*(1), 8862.

Liao, J., Guo, Y., Guo, Z., Zhang, X., Li, X., Kuang, J., Li, J., Bian, X., Wang, W., & Li, J. (2025). In vitro screening of synbiotics based on a four-strain probiotic blend and their therapeutic potential for ulcerative colitis. *Biomedicine & Pharmacotherapy*, *191*, 118521.

Mark, D. G., Hung, Y.-Y., Salim, Z., Tarlton, N. J., Torres, E., & Frazee, B. W. (2021). Third-generation cephalosporin resistance and associated discordant antibiotic treatment in emergency department febrile urinary tract infections. *Annals of Emergency Medicine*, *78*(3), 357–369.

Mboi, N., Syailendrawati, R., Ostroff, S. M., Elyazar, I. R. F., Glenn, S. D., Rachmawati, T., Nugraheni, W. P., Ali, P. B., Trisnantoro, L., & Adnani, Q. E. S. (2022). The state of health in Indonesia's provinces, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet Global Health*, *10*(11), e1632–e1645.

McFarland, L. V. (2021). Efficacy of single-strain probiotics versus multi-strain mixtures: systematic review of strain and disease specificity. *Digestive Diseases and Sciences*, *66*(3), 694–704.

Moghnieh, R., Abdallah, D., Jadayel, M., Zorkot, W., El Masri, H., Dib, M. J., Omar, T., Sinno, L., Lakkis, R., & Jisr, T. (2021). Epidemiology, risk factors, and prediction score of carbapenem resistance among inpatients colonized or infected with 3rd generation cephalosporin resistant Enterobacterales. *Scientific Reports*, *11*(1), 14757.

Montassier, E., Valdés-Mas, R., Batard, E., Zmora, N., Dori-Bachash, M., Suez, J., & Elinav, E. (2021). Probiotics impact the antibiotic resistance gene reservoir along the human GI tract in a person-specific and antibiotic-dependent manner. *Nature Microbiology*, *6*(8), 1043–1054.

Mousa, W. K., Mousa, S., Ghemrawi, R., Obaid, D., Sarfraz, M., Chehadeh, F., & Husband, S. (2023). Probiotics modulate host immune response and interact with the gut microbiota: shaping their composition and mediating antibiotic resistance. *International Journal of Molecular Sciences*, *24*(18), 13783.

Nazir, A., Nazir, A., Zuhair, V., Aman, S., Sadiq, S. U. R., Hasan, A. H., Tariq, M., Rehman,

L. U., Mustapha, M. J., & Bulimbe, D. B. (2025). The Global Challenge of Antimicrobial Resistance: Mechanisms, Case Studies, and Mitigation Approaches. *Health Science Reports*, *8*(7), e71077.

Puljko, A. (2024). *Wastewater as a source for the spread of resistance to third-generation cephalosporins and carbapenems to the aquatic environment*. University of Zagreb. Faculty of Food Technology and Biotechnology ….

Ruef, M., Emonet, S., Merglen, A., Dewez, J. E., Obama, B. M., Catho, G., Andrey, D. O., Kowalski, M., Harbarth, S., & Combescure, C. (2024). Carriage of third-generation cephalosporin-resistant and carbapenem-resistant Enterobacterales among children in sub-Saharan Africa: a systematic review and meta-analysis. *EClinicalMedicine*, *70*.

Sharif, S., Oddie, S. J., Heath, P. T., & McGuire, W. (2023). Prebiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. *The Cochrane Database of Systematic Reviews*, *2023*(6), CD015133.

Silva, H. de O. (2022). *Exploring resistance levels to third-generation cephalosporins and carbapenems and its distribution*.

White, R. T. (2021). Escherichia coli: placing resistance to third-generation cephalosporins and fluoroquinolones in Australia and New Zealand into perspective. *Microbiology Australia*, *42*(3), 104–110.

Zavišić, G., Popović, M., Stojkov, S., Medić, D., Gusman, V., Jovanović Lješković, N., & Jovanović Galović, A. (2023). Antibiotic resistance and probiotics: knowledge gaps, market overview and preliminary screening. *Antibiotics*, *12*(8), 1281.