Comprehensive Investigation of the Therapeutic Efficacy of Lactobacillus Supplementation in Mitigating Antibiotic-Induced Dysbiosis and Toxicity in a Colony of Laboratory Guinea Pigs (*Cavia porcellus*)

Short title: Lactobacillus in Guinea Pigs Antibiotic Toxicity

M. Moharrami[1]; R. Fallahi[2]; M. Eslam Panah[3]

1. Research, Breeding and Production of Laboratory Animals Deptartment, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

2. Research, Breeding and Production of Laboratory Animals Deptartment, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

3. Pathology Deptartment, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran

Abstract

Introduction: Guinea pigs normally have a predominantly Gram-positive intestinal flora. The use of antibiotics causes an imbalance of gram-positive relative to gram-negative bacteria. This leads to acute enterocolitis and then poisoning and death.

Objective: This study was conducted in a laboratory animal breeding facility on a colony of guinea pigs previously treated with antibiotics. Based on clinical and necropsy findings, antibiotic toxicity was diagnosed. Clinical signs included, anorexia, and emaciation, occasionally leading to death. Necropsy findings included congestion in the lungs, liver, kidneys, spleen, and intestines. There was also distension of the gallbladder, cecum, and bladder.

Material and Methods: 48 adult guinea pigs from the affected colony were selected and divided into four experimental groups. Group 1 (control) continued antibiotic therapy. Group 2 discontinued antibiotics. Group 3 received daily probiotic yogurt alongside antibiotics. Group 4 received only probiotic yogurt. Necropsies were performed on deceased or clinically ill animals from the experimental groups and the breeding colony to investigate infectious agents and histopathological changes.

Results: By the fourth week, clinical cases in Groups 1–4 were 50, 30, 25, and 5 percent, respectively, with mortality rates of 100, 50, 35, and 0 percent. Necropsy findings transitioned from hyperacute to mild or normal. Due to the significant reduction in clinical signs and

mortality with probiotic, it was introduced to the colony for four months. By the fourth month, no antibiotic toxicity cases with the aforementioned symptoms were observed in the colony. The disease was completely cured, and no recurrence was detected during at least two reproductive cycles in breeding females.

Conclusion: The use of antibiotics in guinea pigs should be done with extreme caution. The use of yogurt or supplements containing lactobacilli is effective at the same time as and after the administration of antibiotics to help restore the normal microbiota of the guinea pig's intestine.

Keywords: Facillity, Laboratory animal, Poisoning, Treatment, Yogurt

1. Introduction

The gut microbiota is not a static ecosystem but is constantly active and undergoing changes. The gastrointestinal tract harbors approximately 100 trillion microorganisms, predominantly anaerobic, which constitute the microbiota. This number exceeds 10 times the total number of cells in the human body [1]. The gut microbiota comprises a highly complex assembly of diverse microorganisms. Within this intricate system, numerous interactions exist between different microorganisms and between them and the host. Despite this vast diversity, the microbiota rapidly stabilizes into a consistent population. The composition of the microbiota is determined by host and microbial factors. The stabilized microbiota aids the animal in resisting infections, particularly in the gastrointestinal tract [2]. The presence of this microbiota is essential and beneficial for animals. Any imbalance promotes the proliferation of harmful bacteria, adversely affecting animal health and performance [3]. Among the microorganisms constituting the microbiota, lactobacilli inhibit the growth of pathogenic bacteria and play a significant role in the immune system. Dietary changes, antibiotic exposure, and infections may disrupt the symbiosis and balance of the host’s microbiota, leading to the proliferation of pathogenic species and subsequent damage [1]. Guinea pigs typically possess a predominantly gram-positive gut flora. Antibiotics, particularly those targeting gram-positive organisms, can disrupt the balance between gram-positive and gram-negative bacteria. This mechanism leads to acute enterocolitis, toxicity, and death [1, 4]. Guinea pigs are highly sensitive to antibiotics, especially those targeting gram-positive bacteria. The natural intestinal flora of guinea pigs primarily consists of gram-positive organisms such as streptococci and lactobacilli. Administration of anti-gram-positive antibiotics in guinea pigs eradicates the natural flora,

leading to the overgrowth of gram-negative bacteria and opportunistic anaerobes such as *Clostridium difficile* (*C. difficile*). Antibiotics administered orally, injectably, or topically can induce toxicity. *C. difficile* appears to play a primary role in antibiotic-associated enterotoxemia. Additionally, *Escherichia coli* has been observed to cause bacteremia in treated animals. Necropsy of guinea pigs with antibiotic toxicity reveals cecal mucosal edema and hemorrhage. Mucosal necrosis and inflammation may also be seen in the cecum and intestines. All antibiotics should be administered cautiously at the lowest effective dose. Animals often die without clinical signs following antibiotic administration. Those not succumbing acutely may exhibit anorexia, dehydration, and hypothermia prior to death. No specific treatment exists, but avoiding antibiotics effectively prevents this condition [4-7]. Drugs such as penicillin, ampicillin, amoxicillin, chlortetracycline, lincomycin, clindamycin, erythromycin, bacitracin, streptomycin, and cephalosporins can induce toxicity in guinea pigs and should be avoided. These drugs create conditions conducive to the overgrowth of pathogenic species. *C. difficile* overgrowth and its toxin production increase gastrointestinal motility, resulting in diarrhea [5-8]. Broad-spectrum antibiotics such as enrofloxacin, fluoroquinolones, trimethoprim-sulfonamide combinations, tetracycline, and chloramphenicol are considered low-risk for guinea pigs, with minimal potential to harm the microbiota. Oral chloramphenicol at 50 mg/kg every 6–12 hours is non-toxic [4, 9]. Dairy products containing lactobacillus or other probiotics are often recommended to prevent or minimize adverse effects of antibiotic administration in animals such as rabbits, guinea pigs, and hamsters. Additionally, these products are advised for stress-induced diarrhea. Concurrent or post-antibiotic administration of yogurt or dietary supplements containing lactobacilli aids in restoring the natural intestinal flora of guinea pigs. Yogurt can be administered orally at approximately 5 mL daily [5-8]. Fermentative bacteria responsible for dairy product acidification are gram-positive, lactic acid-producing organisms, including lactobacillus, bifidobacteria, and streptococcus. These bacteria compete with potential pathogens for epithelial colonization in the intestines, thereby preventing or minimizing enteritis. Furthermore, lactic acid bacteria are believed to stimulate gastrointestinal immune function, reduce serum cholesterol, and exhibit antitumor properties [6]. Lactic acid probiotics confer health benefits, enhancing weight gain, feed conversion efficiency, gut flora modulation, and disease resistance. Lactobacillus-derived probiotics release protective substances such as enzymes and bacteriocins. They also modify toxin receptors and block toxin-mediated signaling pathways. Lactic acid lowers intestinal pH, enhancing protease, lipase, and amylase activity, thereby improving nutrient digestion and absorption. Probiotic microorganisms can adhere to and colonize the intestinal epithelium [10].

Probiotics are live microbial cells that, when administered in adequate doses, confer health benefits, primarily by reinforcing intestinal and mucosal barriers against enteropathogen colonization, modulating immunity, exerting anticancer and antimutagenic effects, improving lactose utilization, and reducing serum cholesterol. Most probiotics are bacteria of the lactobacillus and bifidobacterium genera, which are components of the gastrointestinal flora. However, non-pathogenic bacteria such as streptococcus, certain *Escherichia coli* strains, *Enterococcus faecium*, and yeasts like *Saccharomyces boulardii* are also used in probiotics. The intestines host a complex and dynamic microbial ecosystem, with microbiota playing critical metabolic, nutritional, and protective roles. The normal structure and function of the intestines result from intricate interactions between the host and resident microorganisms. Several studies demonstrate that probiotics restore mucosal integrity and regulate immune responses [11, 12]. Probiotics are supplements of live microorganisms that, when administered in sufficient doses, benefit the host by balancing the gastrointestinal microbial population. These microorganisms compete with enteropathogens for binding sites in the intestines [3, 11, 12].

2. Materials and Methods

2.1. Study Population and Conditions

This study was conducted in a laboratory guinea pig breeding colony of the Pirbright strain, comprising 180 male and 360 female breeders, along with 400 male and 440 female neonates and juveniles, over a four-month period (from the study's initiation in April to the beginning of September 2023). Clinical signs including lethargy, anorexia, stunted growth, weight loss, emaciation, and reluctance to move were observed, ultimately leading to death. Historical data revealed that the colony had been treated with antibiotics for a bacterial infection approximately six months prior to the study. However, antibiotic use not only failed to control the disease but exacerbated its severity. Clinical cases and mortalities were reported in neonates, adults, and breeders. During the six-month period, enrofloxacin and trimethoprim/sulfadiazine were added to drinking water for the first two months, followed by doxycycline for the subsequent four months in therapeutic doses. Dosages of these drugs were unspecified. Animals were fed standard laboratory guinea pig pellets and provided water *ad libitum*. The breeding system was conventional, using polycarbonate shoebox-type cages (Type 4), with two females and one male per cage. Post-weaning, pups were separated at 200 g body weight, sexed, and transferred to other cages. Sterilized aspen wood shavings were used as

bedding, replaced twice weekly. The breeding room was maintained at 22–24°C, 45–55% humidity, with 8–10 air exchanges per hour (3-minute cycles), a 12:12-hour light/dark cycle, and light intensity below 325 Lux [13].

At the onset of the study, necropsies were performed on deceased or clinically ill guinea pigs suspected of antibiotic toxicity, adhering to ethical guidelines for laboratory animal welfare. To prevent adverse effects of antibiotics, treatment with them was completely discontinued in the colony. Forty-eight adult guinea pigs (16 males and 32 females) were randomly selected from the affected colony and divided into four experimental groups. Animals were housed in compliance with full animal welfare standards and guidelines for the care and use of laboratory animals. Each group was housed in four type 4 cages, with one male and two females per cage. Group 1 (control) continued antibiotic therapy with the same type and dosage as the breeding colony for four weeks. Group 2 discontinued antibiotic administration. Group 3 received probiotic yogurt at 20g/L in drinking water daily alongside antibiotics for four weeks. Group 4 received only probiotic yogurt at 20 g/L in drinking water daily for four weeks. After one month of probiotic yogurt administration in the breeding colony (20g/L in drinking water daily), the regimen was adjusted to three times weekly in the fourth month and once weekly in the fifth month. Necropsies were performed on newly deceased or clinically ill animals from the breeding colony to investigate infectious agents and histopathological changes. Tissue samples (lungs, liver, kidneys, spleen, and intestinal contents) were collected for bacterial culture on Blood Agar, MacConkey Agar, and Tryptic Soy Broth (TSB). Tissue samples for histopathological examination were fixed in 10% formaldehyde solution. Following adequate fixation, 5-µm thick sections were prepared from paraffin-embedded blocks, stained with hematoxylin and eosin (H&E), and examined microscopically. The guinea pigs' diet was sent to a specialized laboratory for chemical and toxicological analysis. Carcasses of euthanized guinea pigs were disposed of using an Infectious waste disposal device (Hydroclave). Statistical analysis of the data (excluding histopathological results) was performed using the Chi-square test, with a significance level set at $P < 0.05$ using the SPSS 27 software.

3. Results

3.1. Clinical observations, mortality rates in symptomatic animals, and necropsy findings in experimental and breeding colonies

By the end of the four-week experimental period, the percentage of the clinical cases observed in groups 1 to 4 were 50, 30, 25, and 5 percent, respectively, and the mortality rates were 100, 50, 35, and 0. The index changes in necropsy signs were hyperacute, acute, moderate and normal, respectively. In the breeding colony, the percentage of clinical cases observed in months 1 to 4 were 30, 15, 5, and 0 precent, respectively, and the mortality rates were 100, 45, 20, and 0, and the index changes in necropsy signs were acute, moderate, mild, and normal, respectively. Clinical signs included lethargy, anorexia, stunted growth, weight loss, emaciation, and reluctance to move, occasionally leading to death.Necropsy findings included congestion in the lungs, liver, kidneys, spleen, and intestines; liver margin swelling; occasional intestinal diarrhea; adrenal enlargement; gallbladder distension; and cecal, intestinal, and bladder dilation (Figure 1).

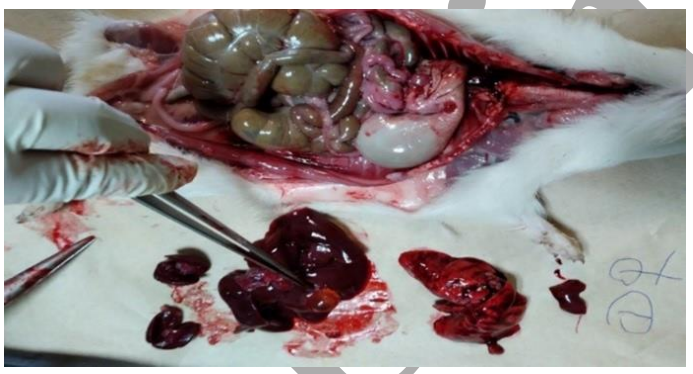

Figure 1. Severe congestion in the lungs, liver, and kidneys; diarrhea in the small intestine; gallbladder and cecal distension.

3.2. Chemical Analysis of the Guinea Pig Diet

Chemical analysis of the guinea pig diet revealed no nutritional deficiencies [14].

3.3. Bacterial Culture Results

Bacterial cultures identified β-hemolytic Streptococcus (pneumoniae, etc.), *C. difficile*, and *Klebsiella pneumoniae*.

3.4. Histopathological Results

Histopathological findings included pulmonary congestion and atelectasis foci; sinusoidal congestion and hemorrhage with hepatocyte ballooning in the liver; renal interstitial edema and tubular coagulation foci; mild to moderate lymphoid depletion and parenchymal edema in the spleen; and submucosal degeneration and edema in the intestines (Figures 2-5).

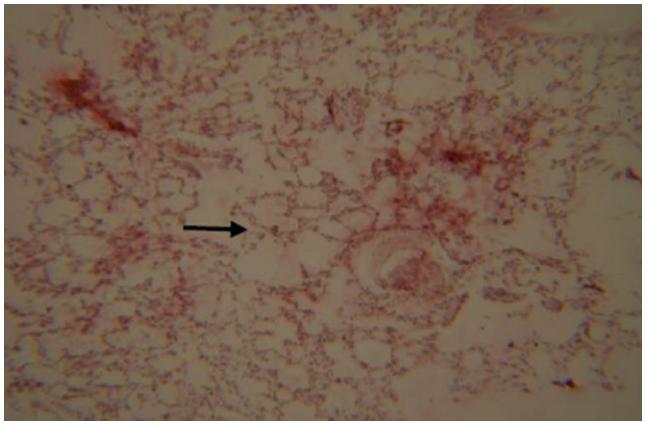

Figure 2. Pulmonary congestion and atelectasis foci (H&E stain, 32x).

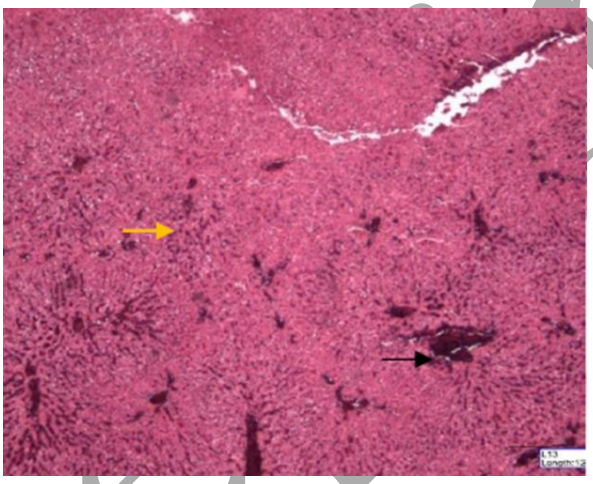

Figure 3. Sinusoidal congestion, hemorrhage, and hepatocyte ballooning in the liver (H&E stain, 100x).

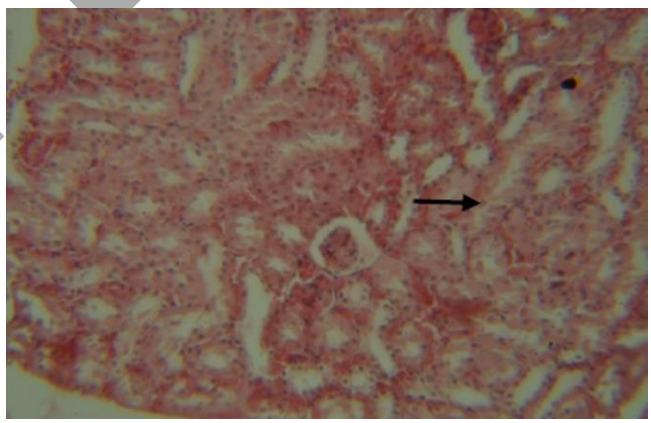

Figure 4. Renal interstitial edema and tubular coagulation foci (H&E stain, 100x).

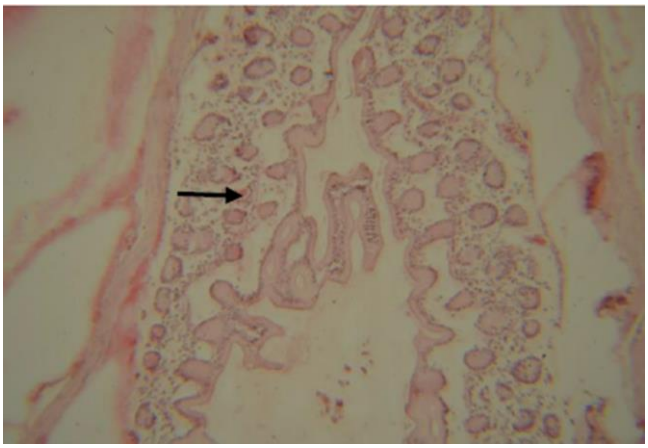

Figure 5. Severe submucosal degeneration and edema in the intestines (H&E stain, 32x).

Following discontinuation of antibiotics and probiotic yogurt administration, a significant reduction in clinical cases, mortality, and histopathological lesions confirmed the resolution of antibiotic toxicity in both experimental and breeding colonies (Figure 6).

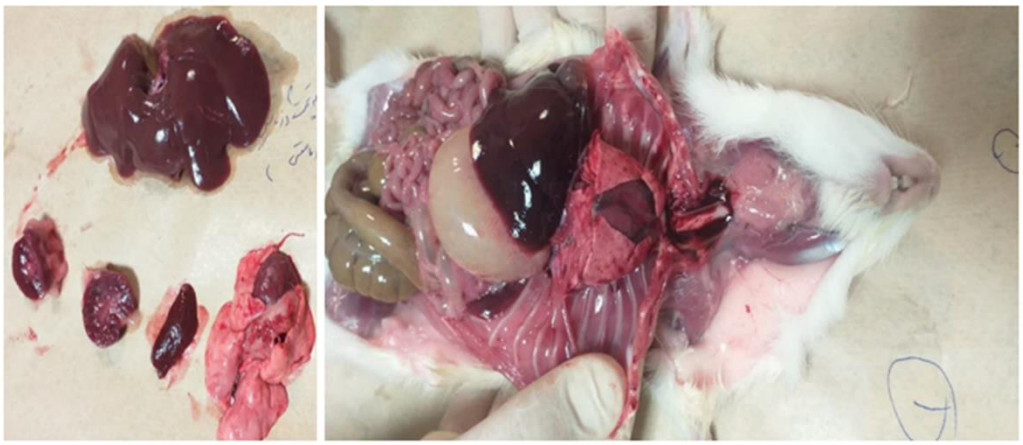

Figure 6. Normal lungs, liver, spleen, kidneys, and gastrointestinal tract at the four month post-intervention.

4. Discussion

Antibiotics are not directly toxic to guinea pigs but induce adverse effects on their gut microbiota. Gram-positive bacteria, particularly Streptococci, are the predominant microorganisms in the gastrointestinal tract of guinea pigs. In the cecum, Streptococci outnumber coliform bacteria by a factor of 100 million. A common side effect of antimicrobial use in certain rodent species, including guinea pigs, is enteritis, with *C. difficile* being the primary causative agent. *C. difficile* is a component of the natural intestinal flora but becomes pathogenic when overgrown, as evidenced by the clinical signs and bacterial culture results in this study. Other factors contributing to antibiotic-associated enteritis in sensitive rodents

include the complex physiology of the intestine and the host's microbiota. Intramuscular administration of 2,000 IU of penicillin or ampicillin at 6 mg/kg kills 75% or more of treated guinea pigs. Goicochea-Vargas, et al. (2025) reported that a single dose of clindamycin phosphate can induce enteritis in guinea pigs [6]. Several antibiotics are implicated in *C. difficile*-associated enteritis in mice, hamsters, and guinea pigs. Spontaneous enteritis caused by this bacterium without antibiotic use has also been reported in rodents. Clinical signs of antibiotic-associated enteritis range from mild diarrhea to acute colitis [6]. The findings suggest that administering yogurt or other lactobacillus-containing products alongside antimicrobial agents prevents or minimizes antibiotic-associated enteritis. Interest in the therapeutic properties of dairy products dates back to Metchnikoff's proposal that daily consumption of fermented milk products enhances health and prolongs human lifespan [5, 6]. Miranda-Yuquilema et al. (2024) reported that agricultural byproducts fermented with lactobacilli and yeasts positively influenced guinea pig gut microbiota, improving intestinal health, weight gain, and reducing diarrhea and mortality while restoring gastrointestinal microbiota to normal levels. In their study, guinea pigs receiving probiotics exhibited increased growth of administered microorganisms (e.g., lactobacillus, saccharomyces) and reduced populations of *E. coli*, Salmonella, and other Enterobacteriaceae [10]. Probiotic use also altered gram-negative and gram-positive bacterial populations, including lactobacilli, bacilli, and yeasts, with significant reductions in pathogenic bacteria such as staphylococcus, enterococcus, listeria, and salmonella. These changes correlated with diminished clinical signs and histopathological lesions, confirming the efficacy of probiotics in restoring microbiota and achieving full recovery [10]. Probiotics are live microbial supplements that, when administered in adequate doses, benefit the host by balancing gastrointestinal microbial populations. These microorganisms compete with enteropathogens for intestinal binding sites, a process enhanced by probiotic-secreted bacteriocins and intestinal peristalsis [3]. Lactobacilli can also combine with nutrients like fiber, exerting therapeutic effects through mucosal adhesion [1]. Gut microbiota and probiotic bacteria produce bacteriocins, organic acids, and hydrogen peroxide, which exert bactericidal effects against enteropathogens. Certain gut bacteria secrete enzymes such as beta-glucuronidases and bile salt hydrolases, releasing bile acids that inhibit undesirable bacteria, while others produce digestive enzymes and metabolites that neutralize bacterial toxins, enhancing intestinal immunity. Probiotics used in animals primarily belong to lactobacillus, streptococcus, lactococcus, and bifidobacterium genera [3]. Criteria for a microorganism to qualify as a probiotic include that it be natural, part of the host's gastrointestinal microbiota and not toxic or pathogenic. Capable of adhering to the host

intestinal epithelium. It should be cultivable on an industrial scale and be stable in commercial preparation. It should not be damaged by the action of digestive enzymes, should quickly settle in the host's intestine, and should have an antagonistic effect on pathogenic microorganisms. It must be stable under storage conditions and in the host animal's body and be able to survive for a long time [2, 3]. Probiotics are employed in poultry, swine, rabbit, cattle, and horse production to improve growth, feed conversion efficiency, and control pathogenic and non-pathogenic microorganisms. Their efficacy depends on animal species, age, health status, probiotic composition, and dosage [3]. Lactobacilli exhibit detoxification properties, neutralizing toxins or toxic compounds critical for host health. For example, *Lactobacillus reuteri* and *L. acidophilus* increase tumor necrosis factor (TNF)-α levels in response to ochratoxin A. In *C. difficile*-associated diarrhea, *L. plantarum*, *L. fermentum*, *L. acidophilus*, and *L. rhamnosus* play therapeutic roles. In this study, probiotic yogurt administration fully resolved diarrhea. Fei et al. reported *L. kefir*'s nitrite degradation and cadmium detoxification activities [1]. Probiotic strains displace pathogens in the host, improving gastrointestinal health. Lactobacilli compete with pathogens for nutrient absorption and mucosal adhesion, secreting antimicrobial agents (organic acids, bacteriocins, hydrogen peroxide) that neutralize pathogens, lower intestinal pH, and produce biosurfactants. *L. acidophilus* and *L. plantarum* inhibit salmonella infection in intestinal epithelial cells. *L. acidophilus* suppresses pathogens such as *Pseudomonas aeruginosa*, *E. coli*, enterobacter, and *Klebsiella spp*. antivirally, lactobacilli block viral entry by coating surface proteins [1]. Gut dysbiosis can lead to diarrhea, enteritis, and colitis. *Bifidobacterium brave*, *B. longum*, *B. infantis*, *L. acidophilus*, *L. plantarum*, *L. bulgaricus*, and *L. casei* mitigate antibiotic-associated diarrhea by restoring microbiota balance [1]. *C. difficile*, a gram-positive, spore-forming anaerobe, causes antibiotic-associated diarrhea and colitis. Boonma et al. demonstrated that vancomycin-resistant *L. rhamnosus* and *L. casei* inhibit *C. difficile*-induced IL-8 production [1]. Probiotics can be administered via various methods, with commercial preparations predominantly containing lactobacillus or streptococcus. Species used include *L. bulgaricus*, *L. acidophilus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. salivarius*, *L. plantarum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *E. faecalis*, *Bifidobacterium spp*., and *E. coli*. Notably, *L. bulgaricus* and *S. thermophilus* (yogurt cultures) qualify as probiotics [2, 15]. The marked reduction in clinical signs, histopathological lesions, and bacterial culture results in this study confirm the efficacy of probiotic yogurt in normalizing guinea pig microbiota and achieving full recovery (Figure 9). Bolla et al. (2013) demonstrated the protective effects of kefir-derived lactic acid bacteria and yeasts against *C. difficile* infection in a hamster model, preventing diarrhea and

enterocolitis [16, 17]. Enterotoxemia refers to the overgrowth of toxin-producing bacteria (particularly Clostridia) in the gastrointestinal tract, exacerbated by stress, abrupt dietary changes, and inappropriate antibiotic use [18]. Clinical signs of antibiotic-associated enterotoxemia emerge 1–5 days post-administration and include anorexia, dehydration, and hypothermia, with variable diarrhea presence. Prevention involves high-fiber diets, stress reduction, and commercial Lactobacillus probiotics [19]. Antibiotic toxicity in rodents and rabbits is a secondary effect of gut dysbiosis. In guinea pigs, mortality often results from toxins produced by *C. difficile* overgrowth (20-24). Colitis severity varies with antibiotic dose, opportunistic pathogen strain, and host susceptibility, manifesting as progressive lethargy, rough coat, diarrhea, and death. Necropsy reveals distended ceca containing bloody fluid, with severe mucosal inflammation and ulceration [9, 18, 23]. *C. difficile* spores persist in the environment, and toxigenic strains produce exotoxins A (enterotoxin) and B (cytotoxin). Toxin B requires toxin A for mucosal access, with toxin A inducing fluid secretion, mucosal damage, inflammation, and cell death [9, 18, 23]. In this study, the significant decline in morbidity and mortality following antibiotic discontinuation and probiotic yogurt use, alongside resolving histopathological lesions, confirms the resolution of antibiotic toxicity in the guinea pig colony (Figure 9). By the fourth month, no antibiotic toxicity cases were observed in the breeding colony, with full disease resolution and no recurrence during at least two reproductive cycles. The colony remained disease-free for 10 months post-intervention, with no issues in vaccine or biological product quality control tests.

5. Conclusion

These findings underscore that probiotics particularly accessible and cost-effective options like probiotic yogurt play a critical role in correcting dysbiosis and accelerating recovery in antibiotic-treated sensitive animals such as guinea pigs and rabbits.

Acknowledgment

The authors thank all the people who provided advice and assistance in this study.

Authors' Contribution

Study concept and design: M. M, R. F.

Acquisition of data: M. M, R. F, M.E.P.

Analysis and interpretation of data: R. F.

Drafting of the manuscript: R. F.

Revision of the manuscript: M. M, R. F.

Critical reversion of the manuscript for important intellectual content: M. M, R. F.

Ethics

The present study was conducted in accordance with the guidelines set by the Animal Ethics Committee of Razi Vaccine and Serum Research Institute, and all experiments were carried out in accordance with relevant guidelines and regulations.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by Razi Vaccine and Serum Research Institute.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

References

1. Di Cerbo A, Palmieri B, Aponte M, Morales-Medina JC, Iannitti T. Mechanisms and therapeutic effectiveness of lactobacilli. Journal of Clinical Pathology. 2016; 69: 187–203.

2. Rabetafika HN, Razafindralambo A, Ebenso B, Razafindralambo HL. Probiotics as Antibiotic Alternatives for Human and Animal Applications. Encyclopedia. 2023; 3:561–581.

3. Carcelen F, Lopez M, San Martin F, Ara M, Bezada S, Ruiz-Garcia L, et.al. Effect of probiotics administration at different levels on the productive parameters of guinea pigs for fattening (*Cavia porcellus*). Open Veterinary Journal. 2021; 11(2): 222–227.

4. Clemons DJ, Seeman JL. The laboratory guinea pig. 2nd ed. CRC Press; 2011.

5. Colby LA, Nowland MH, Kennedy LH. Clinical laboratory animal medicine, An introduction. 5th ed. Wiley Blackwell; 2020.

6. Goicochea-Vargas J, Salvatierra-Alor M, Acosta-Pachorro F, Rondon-Jorge, W, Cajacuri-Aquino J, Herrera-Briceno A, et al. Effects of oral administration of native lactic acid bacteria with probiotic potential on productive parameters and meat quality of fattening guinea pigs (*Cavia porcellus*). Open Veterinary Journal. 2025; 15(4): 1576-1584.

7. Quesenberry K, Orcutt CJ, Mans C, Carpenter JW. Ferrets, Rabbits, and Rodents. 4th ed. Elsevier; 2020.

8. Suckow MA, Stevens KA, Wilson RP. The Laboratory Rabbit, Guinea Pig, Hamster, and Other Rodents. American College of Laboratory Animal Medicine. 1st ed. Academic Press; 2012.

9. Fox GJ, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT. Laboratory Animal Medicine. 3rd ed. Academic Press; 2015.

10. Miranda-Yuquilema J, Taboada J, Once V, Coyago M, Brinez W. Effect of agroindustrial waste substrate fermented with lactic acid bacteria and yeast on changes in the gut microbiota of guinea pigs. Microorganisms. 2024; 12(1): 133.

11. Massi M, Ioan P, Budriesi R, Chiarini A, Vitali B, Lammers KM, et al. Effects of probiotic bacteria on gastrointestinal motility in guinea-pig isolated tissue. World Journal of Gastroenterology. 2006; 12(37): 5987–5994.

12. Kim MJ, Lee YJ, Hussain Z, Park P. Effect of Probiotics on Improving Intestinal Mucosal Permeability and Inflammation after Surgery. Gut and Liver. 2025; 19 (2): 208-218.

13. UFAW. The UFAW handbook on the care and management of laboratory and other research animals. 9th ed. Wiley Blackwell; 2024.

14. National Research Council, Subcommittee on Laboratory Animal Nutrition Committee on Animal Nutrition Board on Agriculture. Nutrient Requirements of Laboratory Animals. 4th revised ed. National Academy Press Washington, D.C.; 1995.

15. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. Cells. 2023; 12 (184): 1-33.

16. Bolla PA, Carasi P, Bolla MdeL, De Antoni GL, Serradell, MdeL. Protective effect of a mixture of kefir-isolated lactic acid bacteria and yeasts in a hamster model of *Clostridium difficile* infection. Anaerobe. 2013; 21: 28-33.

17. Gonzalez-Orozco BD, Garcia-Cano I, Jimenez-Flores R, Alvarez VB. Milk kefir microbiota-Direct and indirect antimicrobial effects. Journal of Dairy Science. 2022; 105(5): 3703- 3715.

18. Mitchell M, Tully TN. Manual of exotic pet practice. 2nd ed. Saunders Elsevier, St. Louis, Mo.; 2019.

19. De Cubellis J, Graham J. Gastrointestinal disease in guinea pigs and rabbits. Veterinary Clinics of North America*:* Exotic Animal Practice. 2013; 16: 421–435.

20. Morris TH. Antibiotic therapeutics in laboratory animals. Laboratory Animals. 1995; 29: 16-36.

21. Narver HL. Antimicrobial stewardship in laboratory animal facilities. Journal of the American Association for Laboratory Animal Science. 2017; 56(1): 6-10.

22. Fallahi R. Antibiotic treatments in laboratory animals. Veterinary medicine and health; The analytical, news, education and information monthly magazine. 2014; 58: 44-56. (In Persian).

23. Turner PV, Wheler CL, Beaufrere H, Fabian N, Harkness JE. Harkness and Wagner's Biology and Medicine of Rabbits and Rodents. 6th ed. Wiley-Blackwell; 2025.

24. Dowling PM, Prescott GF, Baptiste KE. Antimicrobial therapy in veterinary medicine. 6th ed. Wiley Blackwell; 2025.