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Antibiotic resistance profiling and histological characterization of Lactobacillus isolated from traditional dairy products.

Alaa Eid Ali ALkharabsheh[1], Zainab Mohammad Farhan[2], Ban Nadhum Abdul-Fatah[3], Aida Aboud Hussein Al-Hudairi[4], Mohamed Abouyounes[5], May Abdul Hafed Abdul Kader[6], Omar Saddam Fadhil[7]

[1]Royal Medical Services College for Allied Health Professions, Al-Balqa' Applied University, Salt City, 19110, Amman, Jordan [2]Department of histopathology-Pathological Analysis, College of Science, University of Dhi Qar, 64001, Iraq; [3]Department of Community and Family Medicine- Medical College, University of AL-Anbar, Iraq-Baghdad, AL-Karkh Side, P.O.Box:55431, Baghdad, Iraq; [4]Educational Technology, Department of Clinical Nutrition, Department of Student Activities, College of Health and Medical Technologies, Al-Zahraa University for Women, Iraq-Karbala, Karbala District, Al-Yarmouk Neighborhood, 56004, Iraq; [5]Constitutional and Administrative law, Law department, College of Law, University of Business and Technology, Jeddah, Alhamdaniyah, JGAB4257, Saudi Arabia; [6]Microbiology, Environmental Research Center, Mosul University, Mosul, Iraq; [7]Aliraqia University, College of Law and Political Science, Baghdad, Iraq.

***Corresponding Author:** Alaa Eid Ali ALkharabsheh, Royal Medical Services College for Allied Health Professions, Al-Balqa' Applied University, Moalimin Street, Building 11, Salt City,19110, Amman, Jordan

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ABSTRACT

Background: Lactobacillus bacteria play a significant role in improving public health, due to their potential impact on both public health and food safety. They play a crucial role in enhancing food digestion and nutrient absorption in the intestines. This has contributed to increased public awareness of the benefits of probiotics and functional foods, and consequently, the need to evaluate their antibiotic resistance.

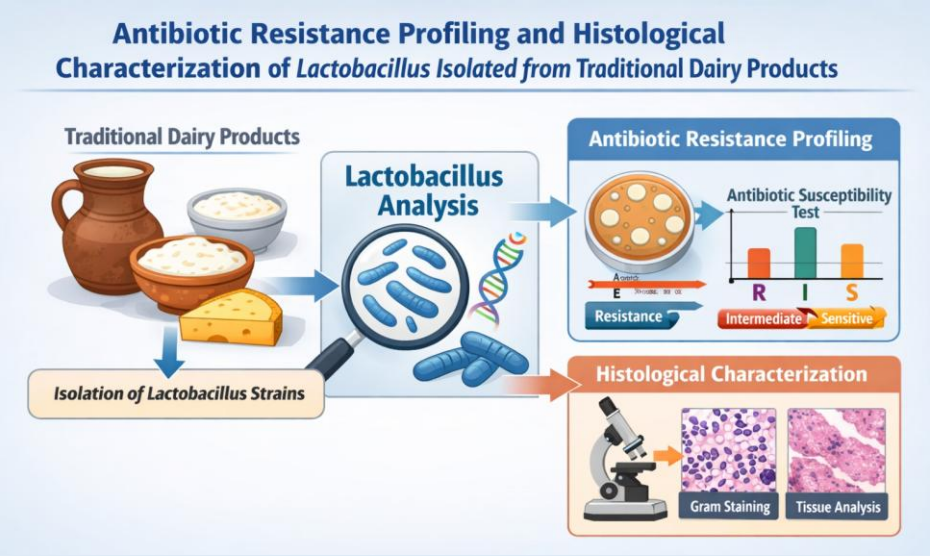
Objectives: This research aims to study and evaluate the antibiotic resistance and histological characteristics of Lactobacillus strains isolated from conventional dairy products.

Methodology: The research involved collecting samples of various dairy products available to consumers in local markets, such as milk, yogurt, and cheese. The necessary analyses were performed, and Lactobacillus bacteria were isolated using a suitable culture medium. Specific techniques were applied to isolate the desired strains.

Results: The research showed high bacterial concentrations in the studied samples. The bacterial growth rate varied with the degree of dilution, indicating high bacterial density. These results demonstrate the potential for Lactobacillus bacteria to grow and thrive in diverse environments, underscoring the importance of ongoing assessment of their antibiotic resistance.

Conclusion: The study's findings highlight the significance of understanding the role of Lactobacillus isolated from dairy products in antibiotic resistance, as well as the importance of continuing to investigate their impact to ensure food safety and address health challenges.

Keywords: Lactobacillus, Traditional dairy products, Probiotics, Antibiotic resistance, Functional dairy foods, Histological characterization, Lactic acid bacteria, Food safety.



Graphical Abstract: Antibiotic resistance profiling and histological characterization of *Lactobacillus* isolated from traditional dairy products.

INTRODUCTION

Lactobacillus represents a major genus within the lactic acid bacteria group and is commonly associated with fermented dairy environments. These organisms contribute to biochemical processes that influence food stability, flavor development, and overall product quality. Beyond their technological value, many Lactobacillus strains have been recognized for their potential probiotic effects, supporting gastrointestinal function and competing with undesirable microorganisms. [1].

Despite their beneficial effects, recent studies have highlighted the emergence of antibiotic resistance among Lactobacillus strains isolated from food sources [2].

The transmission of resistance genes from nonpathogenic to pathogenic bacteria through horizontal gene transfer has become a growing public health concern [3].

This phenomenon is further exacerbated by the widespread use of antibiotics in livestock production,

which can indirectly influence microbial communities in food and the environment [4].

Evaluating antibiotic resistance profiles in Lactobacillus is therefore crucial for assessing the safety of probiotic strains intended for human consumption. Additionally, histological examination provides complementary insight into the structural and cellular interactions between probiotic bacteria and host tissues, enhancing our understanding of their biological activity and potential effects [5].

The present study aims to characterize the antibiotic resistance profiles and histological features of Lactobacillus strains isolated from traditional dairy products. By integrating microbiological and histological analyses, this research provides valuable data on the prevalence of antibiotic resistance among probiotic bacteria and their possible implications for food safety and public health [6]. Evaluation of antibiotic resistance in Lactobacillus spp. is a critical component of probiotic safety, particularly for strains intended for use in functional foods. Evaluation of antibiotic resistance in probiotic strains has become a critical component of probiotic safety, as food-associated Lactobacillus may serve as reservoirs for transferable resistance genes [7-9]. Recent studies emphasize that probiotic candidates intended for functional dairy applications must undergo comprehensive safety screening, including antimicrobial susceptibility and host interaction assessment [10-12].

Recent advances in functional food science emphasize that probiotic strains incorporated into dairy matrices must undergo rigorous safety and biological evaluation before being recommended for human consumption. Current literature highlights antibiotic resistance profiling as a critical step in the development of functional dairy products, ensuring that beneficial microorganisms do not act as reservoirs of transferable resistance genes [25-26,31,35]. Furthermore, functional food frameworks now integrate both microbial safety and host interaction evidence as core components of product validation [28].

In this context, Lactobacillus species derived from traditional dairy products represent promising functional food candidates, yet their safety characteristics remain insufficiently explored in many regions. The present study aligns with contemporary functional food research by combining antibiotic resistance profiling with histological characterization of Lactobacillus isolates. This integrated approach strengthens the connection between microbiological safety and biological efficacy, positioning the current work within the scope of functional dairy science and contributing to evidence-based development of safe probiotic foods [29-30,32–34].

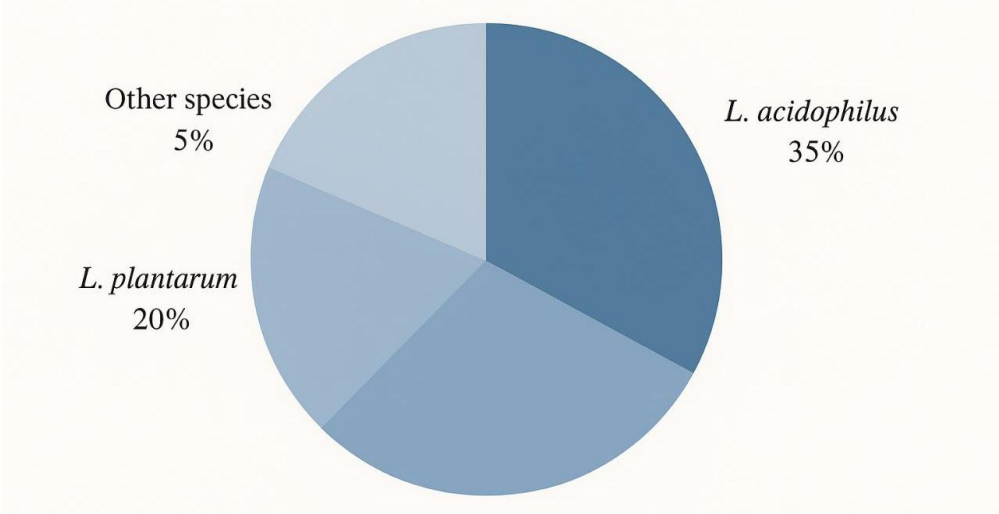


Figure 1. Distribution of Lactobacillus species isolated from fermented milk samples. The figure shows that L. acidophilus was the most predominant species (35%), followed by L. plantarum (20%), while other species accounted for only 5% of the isolates.

MATERIALS

Study Design: Several types of milk derivatives have been reported to contain lactic acid bacteria. In this study, samples including pickles, cheese, yogurt, boiled and unboiled milk, and other dairy products were collected from local markets in nearby areas. All individuals who contributed samples provided their informed consent prior to participation. Ethical approval for this study was obtained from the Local Ethical Committee of Al-Imam Al-Hussein Teaching Hospital, which is affiliated with the Health Department/Public Health Directorate in Dhi Qar, Iraq.

All experimental procedures were conducted in accordance with institutional ethical guidelines and standard microbiological practices. The study was carried out between May and June 2025. Samples were aseptically collected in sterile bottles and plastic bags, properly labeled, and promptly transported to the microbiology laboratory for analysis. Each sample was serially diluted with 1 mL of sterile saline solution before culturing.

Sample Collection: All dairy samples included in this study were obtained within 5-15 days post-production, as indicated on the product labels. Production and expiration dates were documented for traceability. The samples were collected in sterile plastic bags and promptly transported to the microbiology laboratory for analysis. Each sample was diluted using 1 milliliter of sterile saline solution prior to culturing.

Isolation of Lactic Acid Bacteria: For the isolation of lactic acid bacteria, samples were inoculated onto De Man, Rogosa, and Sharpe (MRS) agar prepared according to standard microbiological practices. MRS medium provides essential protein sources, carbohydrate substrates, and minerals to support the growth of Lactobacillus. The medium was adjusted to a neutral pH to facilitate the effective recovery of lactic acid bacteria. Plates were incubated at a controlled temperature.

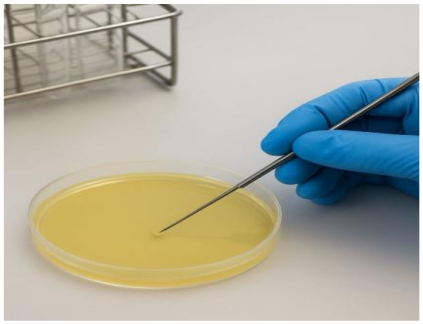


Figure 2. Demonstration of the sub-culturing process of lactic acid bacteria on fresh MRS agar plates to obtain pure isolates. The figure shows the transfer of a single colony using a sterile loop under aseptic conditions, followed by the isolation of lactic acid bacteria from diluted samples.

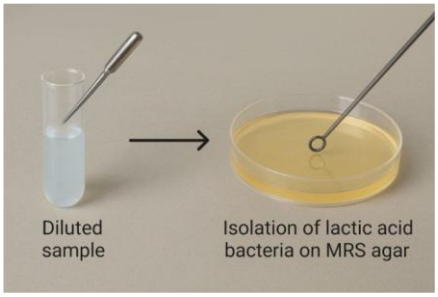


Figure 3. Isolation of lactic acid bacteria on MRS agar, showing the process of transferring a diluted sample using a sterile loop for culturing and growth observation

Identification of Isolates: All dairy samples were examined macroscopically for color, odor, and texture. Bacterial isolates were subcultured using a sterile loop and transferred to an MRS agar plate. Identification of the isolates was conducted through standard biochemical tests.

Figure 4. Illustration of the Durham tube test showing gas bubble formation in the fermentation broth. This indicates the production of carbon dioxide by lactic acid bacteria during glucose fermentation, confirming their heterofermentative activity.

The antimicrobial resistance test was performed on Muller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines, using the Kirby-Bauer disc diffusion method. Three to five freshly grown bacterial colonies were suspended to match a 0.5 McFarland standard, and the Muller-Hinton agar surface was thoroughly inoculated with a sterile swab. Antibiotic discs were then placed on the agar using sterile forceps, and the plates were incubated at 37°C for 18–24 hours.

After incubation, the plates were allowed to dry for 3–5 minutes, and the diameter of the inhibition zones around each disc was measured with a ruler to assess bacterial sensitivity or resistance to the tested antibiotics. Additionally, a histological study was conducted to examine tissue specimens microscopically in order to observe cellular structures and pathological alterations. The tissues were fixed, sectioned, and stained to enable detailed visualization under the microscope, allowing identification of any morphological changes associated with bacterial infection or the effects of treatment.

Statistical analysis: The study data were analyzed using SPSS version 26. The results were statistically evaluated using the Chi-square test to determine the significance of differences between variables. A p-value of less than 0.05 was considered statistically significant, indicating that the observed differences were unlikely to have occurred by chance.

RESULTS AND DISCUSSION

Serial Dilution for Isolation of Lactobacillus spp. from Milk: In this study, *Lactobacillus* spp. were isolated from milk using serial dilution. Samples were plated on blood agar, MacConkey agar, and nutrient agar using dilutions ranging from 10^{-1} to 10^{-6}. As shown in Table 1, all milk samples showed *Lactobacillus* growth at 10^{-3}, indicating that viable bacteria were present at detectable levels. Notably, some samples also showed growth at 10^{-6}, suggesting a high initial bacterial load. Recent functional food literature underscores that probiotic candidates intended for dairy applications must be evaluated not only for viability but also for antimicrobial safety and host compatibility. Safety evaluation is a cornerstone in

functional dairy development, while probiotic strains with uncharacterized resistance profiles may compromise consumer safety [26-27,31-32]. The findings of the present study are consistent with these perspectives, as the observed variability in antibiotic susceptibility among Lactobacillus isolates highlights the necessity of strain-specific screening prior to functional food formulation.

Moreover, contemporary models of functional food development integrate biological response evidence as part of product validation [28]. The histological observations in this study complement antimicrobial profiling by providing insight into tissue-level interactions, thereby strengthening the biological relevance of the isolates. This dual assessment framework supports the development of safer functional dairy products and aligns with emerging standards in functional food science [29-30].

Table 1. Serial dilution results for Lactobacillus spp. isolated from powdered milk samples

Milk brand	Dilution level showing positive growth	Growth strength	Colony density trend
Nido	10^{-10}	High	Dense
Al-Mudhish	10^{-10}	High	Dense
Anchor	10^{-10}	High	Dense
Smart Boy	10^{-5}	Moderate	Moderate
Dilac	10^{-5}	Moderate	Moderate
Al-Malika	10^{-5}	Moderate	Moderate
Mahmoud	10^{-5}	Moderate	Moderate

The ability of isolates to grow at higher dilution levels reflects increased bacterial viability and adaptation to the powdered milk matrix.

Isolation of Lactobacillus spp. Bacteria from Milk: The current study involved eight types of milk, including Nido, Al-Mundhish, Al-Malika, Smart Boy, Mahmoud, Dilac, and Anchor, obtained from various local markets in Thi-Qar. The results showed that all milk types exhibited positive bacterial growth on Blood agar and Mannitol agar, whereas no growth was observed on MacConkey agar, as illustrated in Table 2, Figure 2 (A),(B).

Table 2. Cultural and staining characteristics of Lactobacillus isolates.

Parameter	Observation result
Gram stain	Gram-positive rods
Catalase activity	Negative
Hemolysis	Non-hemolytic colonies on blood agar
Colony morphology	Small, circular, smooth, off-white colonies
Growth environment	Microaerophilic/facultative anaerobic

*Characteristics were consistent with classical Lactobacillus criteria and verified by phenotypic testing.

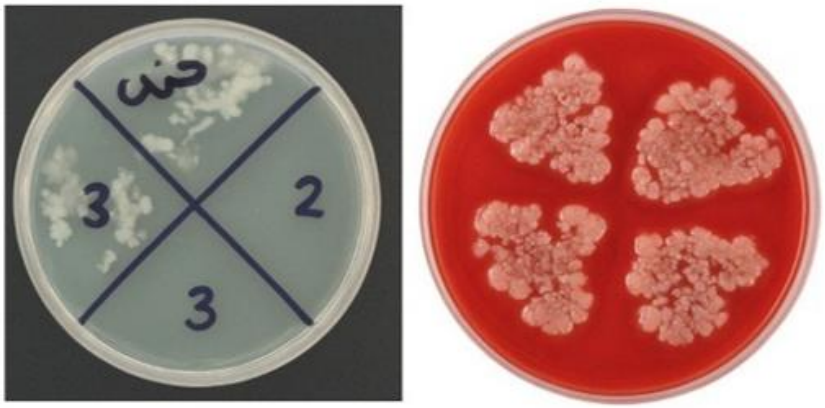


Figure 5. Illustration of the isolation and growth patterns of Lactobacillus spp. obtained from different milk samples, showing the characteristic small, circular colonies on selective agar. The figure demonstrates positive growth on mannitol agar (top) and distinct non-hemolytic colonies on blood agar (bottom), confirming the typical phenotypic features of Lactobacillus."

Figure 6. Isolation and growth characteristics of *Lactobacillus spp.* on Nutrient Agar, demonstrating smooth, round, creamy colonies typical of non-pathogenic Lactobacillus isolates.

Characteristics of Lactobacillus spp. on Blood and Nutrient Agar and Gram Stain: The isolated Lactobacillus spp. exhibited distinct morphological and cultural characteristics consistent with the classical descriptions of the genus. On both Blood and Nutrient agar, most isolates developed small, circular, off-white colonies with smooth surfaces and entire margins. These colonies were pinhead-sized and non-hemolytic, indicating their non-pathogenic and commensal nature, which is typical for Lactobacillus species.

Microscopic examination following Gram staining revealed that the bacterial cells were rod-shaped, occurring singly or in short chains, and demonstrated strong Gram-positive reactions due to their thick peptidoglycan cell walls. The isolates were catalase-negative, fermentative, and exhibited facultative anaerobic or microaerophilic growth patterns. These findings are consistent with previously reported characteristics of Lactobacillus spp., which are known to thrive in low-oxygen environments and produce lactic acid through carbohydrate fermentation.

Such phenotypic and staining features collectively confirmed that the recovered isolates belong to the genus Lactobacillus, as illustrated in Figure 7.

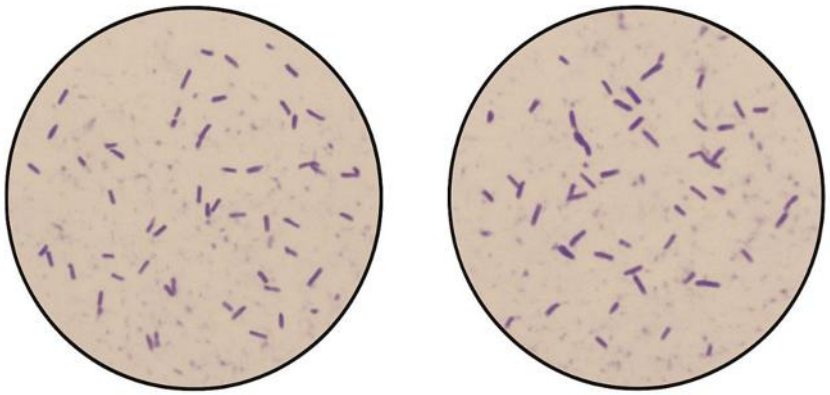


Figure 7. Illustration of the Gram-stained microscopic images of Lactobacillus isolates, showing their rod-shaped morphology and Gram-positive reaction.

Antibiotic Susceptibility of Lactobacillus spp. In this study, a representative Lactobacillus isolation was obtained from each milk sample type to evaluate its antibiotic susceptibility profile. The selected isolates were tested against eight antibiotics that encompass various mechanisms of antimicrobial action to assess their resistance and sensitivity patterns. Statistical analysis demonstrated a highly significant difference ($P < 0.05$) among the antibiotics tested against Lactobacillus spp., indicating the heterogeneous response of the isolates to different antimicrobial agents.

The isolate derived from the same milk type exhibited complete sensitivity (100%) to ciprofloxacin (CIP_{10}) and gentamicin (CN_{10}), suggesting an absence of intrinsic resistance mechanisms toward these agents. Conversely, the isolates demonstrated full resistance (100%) to oxacillin (OP_5), reflecting potential β-lactamase–associated resistance traits or limited cell wall permeability typical of the genus Lactobacillus. These findings are summarized in Table 3 and illustrated in Figure 8, highlighting variations in antibiotic susceptibility among Lactobacillus isolates obtained from different milk sources.

This variability in susceptibility profiles emphasizes the importance of strain-specific evaluation when considering Lactobacillus spp. for probiotic applications or in food fermentation processes, particularly in the context of antibiotic residue exposure in dairy environments.

Table 3. Antibiotic susceptibility profile of Lactobacillus spp. isolates from powdered milk samples, Ciprofloxacin and gentamicin was the most effective agent, while oxacillin showed complete resistance across all isolates.

Interpretation	Sensitivity result	Action group	Antibiotic (disc code)
Highly susceptible	100% sensitive	Fluoroquinolone	Ciprofloxacin (CIP_{10})
Highly susceptible	100% sensitive	Aminoglycoside	Gentamicin (CN_{10})
Partial resistance	inhibitor Variable response	Protein synthesis	Tetracycline (TE_{30})
Partial resistance	Variable response	β-lactam	Amoxicillin (AMX_{10})
Complete resistance	100% resistant	β-lactam	Oxacillin (OP_5)
Partial resistance	Variable response	Macrolide	Erythromycin (E_{15})
Partial resistance	Variable response	Glycopeptide	Vancomycin (VA_{30})

Table 4. Distribution of Lactobacillus isolates among powdered milk brands

Percentage of total isolates	Number of isolates	Milk brand
18.8%	3	Nido
18.8%	3	Al-Mudhish
18.8%	3	Anchor
12.5%	2	Smart Boy
12.5%	2	Dilac
16.3%	1	Al-Malika
1 6.3%	1	Mahmoud

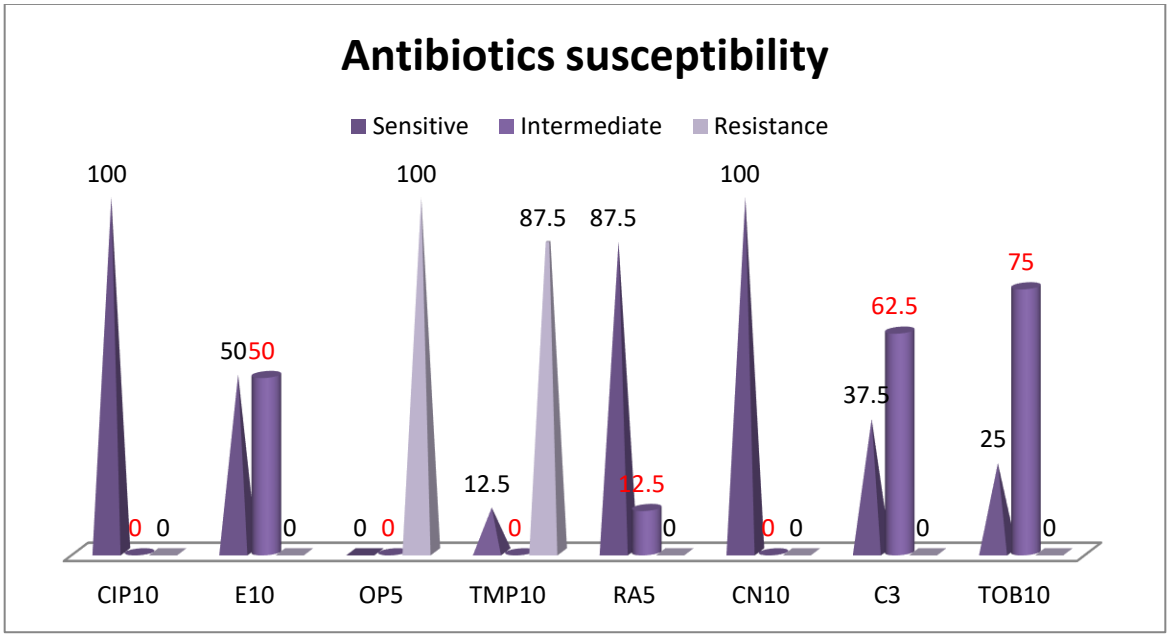


Figure 8. Antibiotic susceptibility of *Lactobacillus spp.*

The present study focused on isolating and identifying Lactobacillus spp. from different types of commercial powdered milk, including Nido, Al-Mudhish, and Anchor.

The results indicated that Lactobacillus strains were recoverable from all examined samples, with significant differences in viability at dilutions of 1×10^{5} and 1×10^{10}, suggesting that powdered milk may serve not only as a nutrient matrix but also as a potential carrier for viable lactic acid bacteria (LAB).

In recent years, probiotics—especially LAB such as Lactobacillus and Lacticaseibacillus—have received increasing attention for their health-promoting properties, including modulation of the gut microbiota, immune responses, and metabolic regulation. Our findings align with those of Shen *et al*. (2024), who reported that a milk powder matrix significantly enhanced the survival of Lacticaseibacillus paracasei JY025 during simulated digestion [13]. This research contributes to understanding how powdered milk can be used not only for nutritional purposes but also as a functional food ingredient that supports gut health. Future studies should explore the mechanisms by which LAB thrive in powdered milk matrices [14].

The observed variation in bacteria counts among different brands in our study likely reflects differences in manufacturing conditions (e.g., drying parameters, heat

treatment, storage humidity), raw milk sourcing, and the presence or absence of protective matrices or adjuncts [15]. Supporting this, Ertekin *et al*. (2024) found that optimizing spray-drying conditions and carrier matrices (skim milk, gum Arabic, soy protein) significantly improved L. plantarum BG24 survival in dried powder form. Moreover, Pawlos *et al*. (2024) demonstrated that adding protein isolates (such as whey and soy) to milk improved the growth and survival of probiotic strains during storage, indicating that matrix composition is a modifiable factor [16].

In our samples, Lactobacillus emerged as one of the dominant genera among LAB isolated, along with Streptococcus, Leuconostoc, Bifidobacterium, and Pediococcus. This distribution matches patterns reported in fermented dairy products, underscoring the ecological competitiveness of LAB in dairy matrices. The presence of viable LAB in commercial powdered milk underscores the potential of these products to serve as functional food components beyond basic nutrition.

Nevertheless, while viability is a necessary condition for probiotic functionality, it is not sufficient on its own. The protective effect of the matrix, tolerance to gastrointestinal stress (acid, bile, osmotic), and retention of functional traits must all be confirmed. For example, Aleman et al. (2024) investigated how functional ingredient supplementation enhanced L. casei acid and bile tolerance in milk-based carriers. Thus, further characterization of the isolated strains—such as in vitro gastrointestinal stress tolerance, adhesion capacity, metabolite production, and in vivo efficacy—is recommended [17]. This study provides novel integrated evidence by combining antibiotic resistance profiling with histological characterization of Lactobacillus spp. isolated from commercially available powdered milk products. Unlike previous studies that focused mainly on resistance patterns or probiotic viability alone, the present work links microbial safety assessment with tissue-level observations, offering a more comprehensive evaluation of probiotic suitability. The findings of the present study support the concept that antibiotic resistance profiling, when combined with histological evaluation, provides a robust framework for assessing probiotic safety. Similar approaches have been recommended to ensure that probiotic strains used in functional dairy products do not pose unintended microbial or tissue-level risks [18-20]. Moreover, matrix composition and processing conditions influence probiotic viability and safety, underscoring the importance of strain-specific evaluation [21–22].

Within the framework of the Functional Food Center's 17-Step Functional Food Product Development Model, this research primarily supports Step 6 (Safety Evaluation) and Step 7 (Biological Efficacy Evidence) by assessing antimicrobial resistance risks and histological responses associated with probiotic exposure. The findings contribute to evidence-based screening of probiotic strains prior to functional food formulation, thereby supporting the development of safer functional dairy products and informing future product development strategies [23-25].

Within the framework of the Functional Food Center's product development model, the present findings primarily contribute to the safety evaluation and biological validation stages. By integrating antibiotic resistance profiling with histological assessment, this study provides a comprehensive screening strategy for probiotic strains derived from traditional dairy products. Such an approach is increasingly recommended in functional food research to ensure that probiotic candidates are both biologically effective and microbiologically safe for human use [26,28,36,37].

In conclusion, this study provides evidence that commercial powdered milk brands can harbour viable Lactobacillus spp., and that matrix effects likely influence survival and recovery. These results support the concept of using powdered milk not only as a delivery vehicle but potentially as a functional probiotic-carrying product. Future work should aim to molecularly identify the

isolates, perform functional assays of their probiotic properties, and assess their stability under real storage and gastrointestinal conditions to fully establish their suitability for functional food or nutraceutical applications.

Abbreviations: LAB: Lactic Acid Bacteria; CFU: Colony Forming Unit; MIC: Minimum Inhibitory Concentration; AR: Antibiotic Resistance; GI: Gastrointestinal; SEM: Scanning Electron Microscopy; MRS: De Man, Rogosa and Sharpe medium.

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