Effect of probiotics on Gram-positive and Gram-negative bacteria that causes different clinical infections

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ABSTRACT

Background: There are more than four known strains of L*actobacillus* bacteria, which have the ability to inhibit and kill antibiotic-resistant bacteria, especially *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This is because it possesses specific mechanisms such as the production and formation of enzymes, acetaldehyde, organic acids, bacteriocins, H_2O_2, etc. Pathogenic bacteria are identified by their morphological characteristics on differential culture media and their ability to ferment lactose and produce mucus and hemolysin. Isolates are diagnosed through biochemical tests with three main tests: catalase enzyme production, oxylase fermentation test, and coagulase test, while molecular diagnosis is performed by using PCR tests and VITEK2 technology. **Results**: The antibiotic-resistant bacteria have the ability to have virulence genes such as *coa, nuc, fimH, hlg*, and *hla*, to name a few, which are widespread in *Staph. aureus* bacteria, and the *hlyA* and *fimH* genes belong to Gram-negative bacteria. *Lactobacillus* bacteria can bind to the host's living tissue cells via adhesion proteins and prevent *Staph. aureus* bacteria from infecting the host's tissues by up to more than 80%, thus maintaining the environmental and life balance within the host and reducing the formation of anti-inflammatory cytokines such as interleukin in particular (IL17), it was also found that *Lactobacillus* in general has the ability to inhibit and kill antibiotic-resistant Gram-negative bacteria (MDA). **Conclusions**: *Lactobacillus strains* can prevent multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria from spreading and multiplying by secreting antigens, proteins, and acidic compounds that disrupt the processes of adhesion of pathogenic bacteria to the living tissues of the host.

Keywords: MDR, Virulence genes, *Lactobacillus*, Gram-positive, Gram-negative.
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INTRODUCTION

Multidrug-resistant (MDR) bacteria are bacteria that are resistant to three or more classes of antimicrobial drugs.

It is considered one of the greatest threats to humanity in terms of health, particularly in developing and impoverished countries. This is due to the frequent use and misuse of antibiotics, which, in turn, lead to the occurrence of genetic mutations in bacterial species, making these bacteria more resistant and aggressive (1).

These bacteria can resist antibiotics due to their mechanisms to protect against toxin secretion and the formation of a capsule or membrane, as well as their virulent genes (2).

Among the most common pathological Gram-positive bacteria resistant to antibiotics is methicillin-resistant *Staphylococcus aureus*, a non-motile spherical bacterium that leads to blood clotting. It is one of the pathogens that infect humans everywhere in the world. It is the most common cause of skin and soft tissue infections, endocarditis, and meningitis of the digestive and respiratory systems (3). *Bacillus*, on the other hand, can produce vesicles that allow it to withstand unfavorable environmental conditions. At the same time, Clostridia bacteria may infect the digestive system and lead to bleeding and ulceration in the colon (4). Many Gram-negative bacteria are resistant to antibiotics, but the most virulent is *Pseudomonas aeruginosa*, which can infect the skin, burns, and wounds.

Escherichia coli, on the other hand, can infect areas of the digestive system (5), and *Klebsiella pneumoniae*, which may be specialized in infecting the respiratory system. However, it is known that the bacteria species mentioned previously can infect other areas of the body if they enter the bloodstream and cause serious diseases such as endocarditis, cerebral meningitis, and septicemia (6).

1- The most critical types of Gram-positive bacteria that are resistant to antibiotics

Staphylococcus aureus is one of the most extensively studied Gram-positive pathogens. Methicillin-resistant strains (MRSA) have evolved resistance to nearly all β-lactam antibiotics due to the acquisition of the *mecA* gene, which encodes an alternative penicillin-binding protein (PBP2a) with low affinity for β-lactams. This resistance extends to penicillins, cephalosporins, and carbapenems, severely limiting treatment options. MRSA is associated with a wide range of infections, from superficial skin and soft tissue infections to life-threatening conditions such as bacteremia, pneumonia, osteomyelitis, and infective endocarditis. In diabetic patients, especially those with chronic ulcers or foot wounds, MRSA poses a serious therapeutic challenge. A study (7) highlighted the prolonged recovery time and high recurrence rate in MRSA-infected diabetic foot ulcers, which are often complicated by biofilm formation and immune evasion. The spread of MRSA within healthcare settings is a critical concern. Nosocomial MRSA strains are commonly transmitted through contact with contaminated surfaces, equipment, or healthcare personnel. A study (8) emphasized the need for rigorous infection control measures, including contact precautions, antimicrobial stewardship programs, and environmental decontamination, to reduce MRSA incidence in hospitals and long-term care facilities.

Although traditionally classified as a non-pathogenic environmental organism, *Bacillus subtilis* has recently emerged as an opportunistic pathogen, particularly in immunocompromised individuals. Studies (9) have identified several virulence-associated genes in clinical isolates, including those encoding hemolysins, proteases, lipases, and enterotoxins—enzymes and toxins that contribute to tissue degradation, immune evasion, and gastrointestinal disturbances. PCR-based analyses have enabled the detection of specific virulence genes, such as *aspHS*, which may vary in expression depending on host factors like gender, immune status, and comorbidities. Although *B. subtilis* infections remain relatively rare, their potential to harbor and express virulence determinants, especially in nosocomial environments, underscores the importance of genomic surveillance and molecular diagnostics.

Members of the Genus *Clostridium*—notably *C. difficile*, *C. perfringens*, and *C. botulinum*—are spore-forming, obligate anaerobes that cause a broad spectrum of diseases. These organisms are notorious for their ability to produce potent exotoxins, such as TcdA and TcdB in *C. difficile*, alpha-toxin in *C. perfringens*, and botulinum neurotoxins in *C. botulinum*. These toxins disrupt host cellular processes, cause necrosis, and can lead to systemic toxicity. *Clostridium* species are also highly resistant to adverse environmental conditions due to their ability to form endospores, which can remain viable through extreme heat, desiccation, and disinfectants. A study (10) demonstrated that *Clostridium* spores can survive typical cooking temperatures and proliferate in inadequately stored or reheated food, particularly in meat, poultry, and broth-based products. Outbreaks of foodborne clostridial illness often arise from such conditions, with symptoms ranging from mild diarrhea to severe colitis and intestinal necrosis. Multidrug resistance in *Clostridium* spp. includes resistance to macrolides, clindamycin, and fluoroquinolones, often mediated by ribosomal methylation (e.g., *erm* genes), efflux mechanisms, and enzymatic drug modification. *C. difficile*, in particular, has shown increasing resistance to metronidazole and vancomycin, first-line treatments for colitis, prompting interest in novel therapeutics, such as fecal microbiota transplantation (FMT) and bacteriophage therapy.

2- The most essential types of resistant Gram-negative bacteria to antibiotics

Pseudomonas aeruginosa is considered one of the most dangerous Gram-negative bacteria and could be multidrug-resistant (11).

Another virulent, antibacterial-resistant bacterium is *Klebsiella pneumoniae*. A study conducted on blood samples from newborns suspected of having bacterial septicemia found that 23 out of 125 blood samples were infected with Gram-negative bacteria, with *K.pneumoniae* being the most prevalent species. It was 65% in males and 35% in females (12). Genetic analysis of *K. pneumoniae* has identified the presence of *the oqxa gene* and *oqxb*, which may contribute to reducing bacterial susceptibility to multiple antimicrobial agents (13).

Additionally, *Escherichia coli*, especially the most common cause of diarrhea, enteropathogenic *E. coli* (EPEC), are found in many strains. It is associated with long-term intestinal colonization, chronic intestinal wall inflammation, and weight loss. Studies consistently report that it exhibits multidrug resistance (MDR), which may be attributed to the presence of virulence genes. (14-15).

Several studies have shown that *Escherichia coli* (*E. coli*), *P. aeruginosa*, and *K. pneumoniae* exhibit complex and diverse resistance patterns against various classes of antibiotics due to their possession of multiple resistance mechanisms. For instance, these bacteria produce β-lactamases such as AmpC and extended-spectrum β-lactamases (ESBLs), which inactivate penicillins and cephalosporins. Some strains also produce carbapenemases of classes A, B, and D, contributing to resistance against carbapenem antibiotics, often considered last-resort treatments. In addition, these pathogens utilize efflux pumps and target site modifications (e.g., mutations in DNA gyrase or RNA polymerase genes) to resist fluoroquinolones and rifampicin. Resistance to aminoglycosides is often mediated by modifying enzymes or, in the case of *K. pneumoniae*, by 16S rRNA methylation. This broad range of resistance mechanisms underscores the urgent need for alternative therapeutic strategies, such as the application of probiotic-derived products or antimicrobial peptides, particularly in the face of rising multidrug-resistant (MDR) bacterial infections Table (1).

Table (1): The most essential mechanisms of antibiotic resistance in Gram-negative bacteria (16)

Type of antibiotic	***E. coli***	***P. aeruginosa***	***K. pneumoniae***
Penicillins	Amp C, ESBLs, other b-lactamases	Amp C, ESBLs, other b-lactamases	Amp C, ESBLs , other b-lactamases
Cephalosporins 1st gen.	Amp C, ESBLs	Amp C, ESBLs	Amp C, ESBLs
Cephalosporins 2nd gen.	Amp C, ESBLs	Amp C, ESBLs	Amp C, ESBLs
Cephalosporins 3rd gen.	Amp C, ESBLs	Amp C, ESBLs	Amp C, ESBLs
Cephalosporins 4th gen.	ES BLs	ES BLs	ESBLs
b-lactamase inhibitors	Amp C	Amp C	Amp C
Aztreonam.	ESBLs	ESBL s	ESBLs
Carbapenems.	Class A, B & D carbapenems mases	Class B and D carbapenem mases	Class A, B, and D carbapenem mases
T, tetracyclines	Efflux pumps	Efflux pumps	Efflux pumps
Tigecycline	Efflux pumps, porin downregulation	Efflux pumps	Acr AB efflux pump
Macrolides and clindamycin	Efflux pumps, macrolide	Efflux pumps	
Fluoroquinolones	Mutations in the DNA gyrase gene	Mutations in topoisomerase IV and DNA gyrase genes, efflux pumps	Mutations in the DNA gyrase gene, efflux pumps, enzyme protection proteins, and degradation enzymes
Rifampicin	Mutations in the RNA polymerase gene	Mutations in RNA	Enzymatic degradation
TMP/SMX	Overproduction of DHFR, mutation	Efflux pumps	
Aminoglycosides	Aminoglycoside degradation enzymes	Aminoglycoside degradation enzymes, efflux	Aminoglycoside degradation enzymes, production of 16*SrRNA*

Abbreviations: ESBLs, extended-spectrum β-lactamases; TMP/SMX, trimethoprim–sulfamethoxazole; DHPS, dihydropteroate synthase; DHFR, dihydrofolate reductase; AmpC, AmpC β-lactamase; β-lactamases, β-lactamases; Carbapenemases (Class A/B/D), Carbapenem-hydrolyzing β-lactamases; AcrAB-TolC, AcrAB-TolC efflux pump system; TMP/SMX, trimethoprim-sulfamethoxazole; 16S rRNA, 16S ribosomal RNA.

3-The inhibitory effect of *Lactobacillus* on some types of Gram-positive and Gram-negative bacteria

Lactobacillus bacteria have been used to replace preservatives in the food industry because they can inhibit the growth of antibiotic-resistant bacteria and those that cause food poisoning (17).

According to a study by (18), the inhibitory effectiveness of the probiotics examined on different types of Gram-positive and Gram-negative bacteria was evaluated using a antimicrobial susceptibility test (AST). The study's data confirmed excellent inhibitory activity for both Bifidobacteria and the mixture of *Acidophilus, Lactobacillus, and* Bifidobacteria. By measuring the inhibition zone, it was found that probiotics have a good and practical inhibitory effect on both Gram-positive bacteria (*Streptococcus*) and Gram-negative bacteria (*Proteus* and *Pseudomonas*). Additionally, it was found that there is an inhibitory effect for *Lactobacillus* and *Bifidobacterium* when tested individually, and there is a synergistic effect when they are mixed (MBL), as shown in (Figure 1).(17)

A study conducted in Iraq showed that *L. plantarum* has a protective effect on the intestine of mice infected with *Salmonella typhimurium* (19). Another study demonstrated that bacteriocins produced by *Lactobacillus* have inhibitory effects on certain pathogenic bacteria that infect the female reproductive system (20). Studies have also shown that *L. plantarum* effectively inhibits *Staph. aureus and P. aeruginosa* (21). Furthermore, studies have shown that glycolipids produced by *L. plantarum can inhibit Staph. aureus and P. aeruginosa* (22). A study by (23) also demonstrated that the *Lactobacillus* genus affects reducing cholesterol levels in mice.

In a study by (17), lactic acid bacteria were found to produce (PlnEF), which is a two-component class IIb bacteriocin that has potent antimicrobial activity against some Gram-positive bacteria. It was also found that lactic acid produced by *Lactobacillus* bacteria has an inhibitory effect on Gram-negative bacteria like *Aeromonas hydrophila*, and it was also shown that there is a synergistic inhibitory effect. A synergy was found between PlnEF and lactic acid produced by *Bacillus* bacteria against Gram-negative bacteria and potential pathogens, *A. hydrophila* LPL-1. It is naturally resistant to PlnEF. The study also confirmed that lactic acid led to the release of the LPS compound against *A. hydrophila*, which enabled the PlnEF compound to contact the inner cell membrane of *A. hydrophila* bacteria. Ultimately, the combined treatment of lactic acid and PlnEF together led to severe external and internal morphological changes in *A. hydrophila* bacteria, including the appearance of bubbles on the external cell surface, abnormal cell elongation, disruption of its internal membrane, and the formation of pores through the outer and inner bacterial membrane also causes coagulation of the cytoplasm. Mutation and change of the actual structure of DNA and protein profile analysis revealed that co-treatment of PlnEF and lactic acid inhibits energy synthesis, protein synthesis, and protein replication, thus preventing DNA replication in *A. hydrophila*. Finally, these results showed that lactic acid with PlnEF was very effective against *A. hydrophila* bacteria and proved that it used these mechanisms to inhibit and kill multidrug-resistant bacteria, including *A. hydrophila* bacteria.

4- Methods of using *Lactobacillus* to inhibit Gram-negative and Gram-positive bacteria

One of the most widely used types of probiotics is lactic acid bacteria (LAB), which can inhibit and kill many Gram-negative and Gram-positive pathogens. It is known that the most essential substances produced by LAB bacteria include enzymes, acetoin, acetaldehyde, organic acids, bacteriocins, and hydrogen peroxide (H_2O_2) (24).

Many studies have found that compounds produced by LAB bacteria are more effective in inhibiting and killing Gram-negative bacteria (25). Gram-negative bacteria employ a crucial method to prevent antibiotics from penetrating their cell membranes (OM) (26). This is where lactic acid plays a role, as it disrupts the OM barrier, allowing inactive antimicrobial agents to penetrate the bacterial cells and kill the bacteria (27). The use of hydrophobic antibiotics, detergents, lysozyme, polyanionic polyethyleneimine, polymyxin, and protamine may kill or inhibit all types of Gram-negative bacteria in the presence of lactic acid (28).

The study (29) demonstrated that a synergy exists between the first type of nisin-producing bacteria and lactic acid, which inhibits Gram-negative pathogens such as *P. aeruginosa* ATCC 9721 and *Pseudomonas fluorescens.* It was found that this synergy results from acid stress (40% lactic acid, 16% acetic acid, and 16% propionic acid, specifically in water), and this increases the sensitivity of bacteria, *P. fluorescens* PF2 and *Yersinia enterocolitica* Y7P, to the compounds pediocin and nisin. Ultimately, it became clear that the synergistic activity of pediocin-type bacteria leads to the failure of the proton motive force at the inner cell membrane and the death of the bacterial cell.

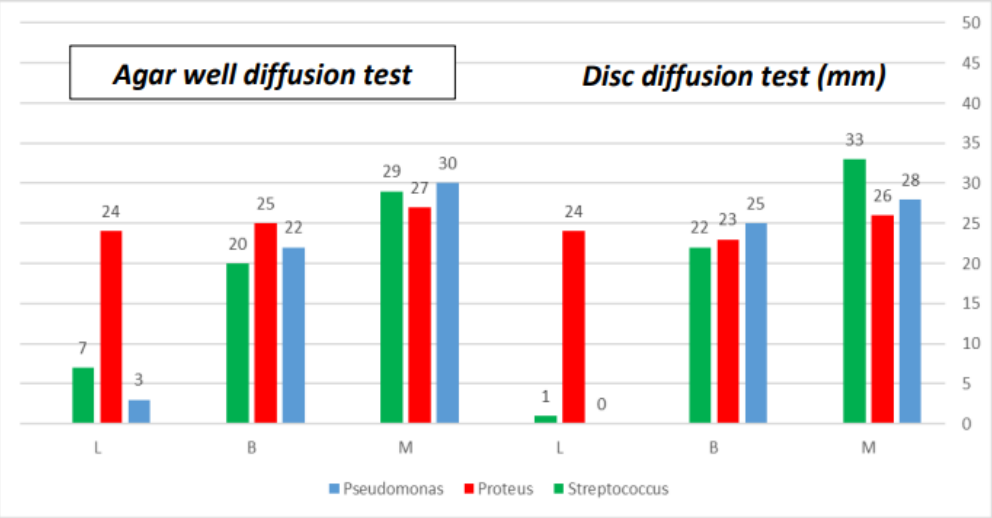

Figure (1): The effect of probiotics *Bifidobacterium* (B), *Lactobacillus* (L), and the mixture (M) on bacterial study isolates (17).

5- Virulence genes in Gram-positive bacteria

Adhesive proteins, toxins, and cell surface proteins, in addition to enzymes, are virulence factors that play a critical role, and the heterogeneity in the composition and severity of these diseases significantly influences their pathogenesis and therapeutic approaches (30). Susceptibility testing was performed using 26 different antibiotics (31). Using PCR technology to detect the presence of virulence genes, *finbB, clfA, efb, icaA, pvl, agr, spa, coa, nuc, hla, finbA, sdrC,* and *icaA* genes were detected in most of the isolates under study. It has been shown that there is a clear relationship between virulence genes and resistance to antibiotics or multiple drugs. It has become clear that the highest resistance was in isolates containing the following genes: *sdrC, sdrD, clfA, icaA, coa, efb, finbB, nuc, hlg, and finbA.* There were significant relationships between *Staph. aureus* and MRSA, and the presence of the *sdrD, icaA, coa, nuc, clfA, etb, finbB, sdrC, hlg,* and *finbA* genes at a probability of error of 5%. The results also showed the presence of the *plaZ* and *mecA* genes among all bacterial isolates. A study (32) showed that 6.13% of the bacterial isolates were diagnosed as *Staph. aureus* in the infected samples that were gathered during isolation. 29% of the infected people had five types of isolates in the blood, and 32% of the isolates produced biofilms. (7.8%) the isolates contain the *fimH* gene, which belongs to the Gram-negative bacteria *Escherichia coli.* (9.2%) of them hide the *icaA* gene, which belongs to Gram-positive bacteria (6.4%) of the bacterial isolates belonged to *Staphylococcus* spp, bacteria that hide the *HLA* gene. In addition, (5.08 %) of the samples had the *hlyA* gene, which belongs to Gram-negative bacteria. It has been found that infectious organisms can be transmitted from one person to another by contagious substances responsible for acquired infections. Table (2)

Table (2): Displays some virulent genes in various types of multidrug-resistant (MDR) bacteria (32).

Target gene	Type of microorganism	Responsible characteristics	Percentage
icaA	Gram +ve. bacteria (S. *aureus*)	Regulate the production of Exopolysaccharide (ESP) in biofilm	9.2 %
Hla	Gram +ve bacteria (*S. aureus*)	Producing: hemolysin	6.4 %
hlyA	Gram -ve bacteria (*E. coli*)	formation of toxin & cause damage in tissue & promote inflammatory cytokines	5.08 %
fimH	Gram -ve (*E. coli* and *P. aeruginosa*)	Regulate the production of Exopolysaccharide (ESP) in biofilm	7.8%

6- The inhibition effect of LAB on the adhesion capacity of Gram-positive and Gram-negative bacteria to host cells.

The results of a study (33) showed that *L. delbrueckii* 45E can bind to HeLa cells, which are cells that imitate uterine cells, thereby reducing the ability of pathogenic bacteria, such as *Staph. aureus***,** to adhere to the surfaces of vital tissue cells and maintain environmental balance. *L. delbrueckii* 45E also showed that a strong adhesion to HeLa cells of about 86% of Ld45E was able to bind to HeLa cells at the original concentration of 5×10^7 CFU/mL and 4.3×10^7 CFU/mL. The control group, which is *L. reuteri* RC-14, showed low adhesion, estimated at equivalent (64%), with a recovery of 3.2×10^7 CFU/mL. Also, *L. delbrueckii* 45E was sensitive to the antibiotics (beta-lactam ceftriaxone) (30 µg) and (Cefuroxime) (30 µg). Pretreatment with the antibiotic and inhibitory protein of HeLa cells using strain *L. delbrueckii* 45E significantly inhibited IL-17 production and synthesis in response to *E. coli* 0.114 OD450 nM and (250 pg/ml) C. parapsilosis equivalent to (31.25 pg/ml). No significant decrease in IL-17 production was observed with GBS compared to the control group of HeLa cells not treated with *L. delbrueckii* 45E bacteria, as shown in Table (3).

Table (3): Inhibition rate of *L. delbrueckii* 45E bacteria against the selected group of pathogenic bacteria (33)

Species	***Streptococcus* clinical isolate**	***Streptococcus* ATCC 8017**	***E. coli* clinical isolate**	***E.coli* ATCC 25,922**	***Klebsiella* spp. clinical isolate**	***Klebsiella* spp. ATCC3566**
L. delbrueckii 45E	50.3 ± 1.9 *	50.2 ± 2.0*	30.2 ± 0.4	30.2 ± 0.4	25 ± 0.2	24.1 ± 0.2
P value	0.001	0.001	0.12	0.12	0.08	0.09
L. reuteri RC - 14 G. control	22.3 ± 0.8*	24.2 ± 1.1*	18.4 ± 0.7	18 ± 1.9	18 ± 0.9	16.9 ± 0.09
P value	0.01	0.01	0.09	0.09	0.49	0.45

The result was based on the average values of three independent examinations, with the arithmetic mean data represented as the mean ± standard deviation (SD).

*** Shows significant OR, $p < 0.05$ compared to the control group, *L. reuteri* RC-14.**

The putative bacteriocin genes, along with the probiotic capabilities of *Lactococcus* and *Lactobacillus* species, particularly their roles in maintaining immune homeostasis and preserving the integrity of the intestinal epithelium, are of significant importance. These bacteria are known to produce high levels of gamma-aminobutyric acid (GABA). They can inhibit the adhesion of intestinal pathogens such as *Salmonella intestinalis* and *E. coli* to Caco-2 epithelial cells. Notably, *Lactococcus garvieae* KS1546 has been shown to produce a bacteriocin named garvicin, which exhibits antimicrobial activity against various pathogenic bacteria. Through the application of genetic engineering techniques and optimization of growth media and culture conditions, the production of garvicin by

strain KS1546 was enhanced by 2000-fold. The modified bacteriocin (referred to as M2) demonstrated broadened antimicrobial activity, including effectiveness against Gram-negative bacteria. This inhibitory effect is attributed mainly to the presence of 1-acetyl-β-carboline, a bioactive compound with documented antitumor, antiviral, antibacterial, and antiparasitic properties. Additionally, *Bacillus* sp. M2 produces other bioactive metabolites such as bacillomycin and surfactins, which possess fungicidal properties and contribute to the control of a wide range of pathogenic microorganisms. (34)

Discussion

Numerous studies have demonstrated that specific *Lactobacillus* isolates exhibit strong inhibitory activity against both Gram-positive and Gram-negative multidrug-resistant bacteria. *Lactobacillus* species employ multiple mechanisms to suppress pathogenic bacterial growth, including the expression of antimicrobial genes Table (3) and the production of class IIb bacteriocins—two-component peptides synthesized by lactic acid bacteria. One such bacteriocin, PlnEF, exhibits potent inhibitory effects against *Staph. aureus* by binding to its cell wall and downregulating several key virulence genes, including *sdrD*, *icaA*, *coa*, *nuc*, *hlg*, and *hla*. This interference leads to significant morphological and structural alterations in *Staph. aureus*, such as membrane blebbing, abnormal elongation, disruption of the inner membrane, pore formation, and cytoplasmic coagulation at the cell surface. Additionally, the bacteriocin Garvicin, produced by *Lactococcus garvieae* strain KS1546, has shown activity against antibiotic-resistant *Staph. aureus*. By optimizing culture conditions through media modification, genetic engineering, and fermentation strategies, Garvicin production in strain KS1546 was enhanced by up to 2000-fold. This increased production correlates with the inhibition of *Staphylococcus* adherence to host cells, as previously reported in the literature (35).

Studies have demonstrated that *L. delbrueckii* 45E can adhere to *Helicobacter* cells that mimic uterine epithelial tissue, thereby limiting the attachment of pathogenic bacteria to host cell surfaces and contributing to the maintenance of mucosal homeostasis. Notably, *L. delbrueckii* 45E exhibited an 86% adhesion rate to HeLa cells, significantly higher than the 68% observed in the control group. This strong adhesive capability is attributed to the production of specific bioactive compounds, which also contribute to the downregulation of interleukin-17 (IL-17), a cytokine involved in inflammatory responses and antimicrobial defense. In contrast, when evaluating the immune response to *K. pneumoniae* infection using a control group of *Bacillus* sp. GBS, no reduction in IL-17 levels was observed, with an optical density (OD_{450}) measurement of 0.257 (33).

In addition, certain *Lactobacillus* strains, particularly their cell-free supernatants (CFS), have demonstrated inhibitory effects against pathogenic bacteria such as *Bacillus* and *Clostridium* species, which are commonly associated with foodborne illnesses. The antimicrobial activity of *Lactobacillus* can be attributed to their ability to secrete a variety of bioactive substances, including organic acids (e.g., lactic acid and acetic acid), hydrogen peroxide, ethanol, diacetyl, bacteriocins, and antimicrobial peptides. These metabolites play a crucial role in suppressing the growth of disease-causing microorganisms and offer potential as alternative therapeutic agents, pending the identification and characterization of their active components. Experimental studies have confirmed the efficacy of these metabolites against both Gram-positive and Gram-negative pathogens. For instance, *Staph. aureus* was found to be highly sensitive to a specific *L. plantarum* strain, exhibiting an inhibition zone of 20 mm, while *B. cereus* showed no significant sensitivity. This antimicrobial activity has been linked to *L. plantarum* and another strain, HM-2, both of which were isolated from human milk. Other Gram-positive pathogens, such as *Strep. mutans*, also displayed moderate sensitivity to *L. plantarum* and *Lactobacillus brevis*. Notably, *S. aureus* strains showed high susceptibility to CFS from *Lactobacillus rhamnosus* and *Lactobacillus casei*, findings that align with other studies (36–37).

According to numerous studies, *Lactobacillus* strains generally exhibit greater inhibitory activity against Gram-negative bacteria compared to Gram-positive bacteria. This differential effect is attributed mainly to the structural characteristics of bacterial membranes. Specifically, Gram-negative bacteria possess an outer membrane (OM) and a thinner peptidoglycan layer, making them more susceptible to disruption by antimicrobial compounds produced by *Lactobacillus* and *Corynebacterium* species. In contrast, the thicker peptidoglycan layer in Gram-positive bacteria contributes to their relatively higher resistance. This study supports the hypothesis that interference with capsular polysaccharide (CAP) function and the outer membrane integrity is a key mechanism by which *Lactobacillus* exerts its antimicrobial effects. Moreover, findings indicate a notable inverse relationship between *Lactobacillus* abundance and the presence of Gram-negative bacteria in the context of bacterial vaginitis. Individuals with this condition show a marked increase in Gram-negative bacterial populations and a corresponding decline in *Lactobacillus* levels. Conversely, in healthy women, urine samples typically reveal lower counts of Gram-negative bacteria and elevated levels of *Lactobacillus* bacilli. These beneficial bacteria may exert their effects through various mechanisms, including inhibition of cell wall and cytoplasmic membrane synthesis, disruption of protein synthesis and DNA replication, alteration of bacterial metabolism, or interference with antibiotic resistance pathways. These findings are consistent with previous reports (38).

Similar to Gram-positive bacteria, Gram-negative bacteria also exhibit variable sensitivity to different *Lactobacillus* strains. For example, *L. brevis* demonstrated no inhibitory effect on Gram-negative species such as *E. coli* and *K. pneumoniae* when tested using cell-free supernatants (CFS) from six different *Lactobacillus* strains.

Among the six *Lactobacillus* cell-free supernatants (CFS) tested, only two demonstrated inhibitory activity against *Proteus mirabilis* and *P. aeruginosa*. Additionally, strains such as *L. rhamnosus* GG and *Lactobacillus casei* exhibited strong antimicrobial effects against pathogenic Gram-negative bacteria, including *Salmonella typhi*, *Salmonella flexneri*, and *P. aeruginosa*. In contrast, commercially available bacteriocins showed potent inhibitory activity primarily against Gram-positive bacteria, with moderate efficacy against *Strep. mutans*, *Staph. aureus*, and *B. cereus*. One of the most widely used commercial bacteriocins demonstrated limited activity against Gram-negative bacteria, likely due to its physicochemical properties and inability to effectively penetrate the outer membrane of these organisms. Conversely, *Lactobacillus* CFS exhibited strong inhibitory effects against the same Gram-negative pathogens, suggesting a broader spectrum of action likely driven by their complex mixture of bioactive compounds. These findings align with previous research, including Study (36), which reported that a synergistic combination of CFS derived from *L. casei* and *L. rhamnosus* was particularly effective against *P. aeruginosa*, a highly drug-resistant and opportunistic Gram-negative pathogen. Infections caused by *P. aeruginosa* are increasingly challenging to manage due to their multidrug resistance and association with high morbidity and mortality. The antagonistic activity of *Lactobacillus* CFS against *P. aeruginosa* underscores their potential as alternative or adjunctive agents in combating antibiotic-resistant infections.

In addition to their direct antimicrobial effects, the presence of *Lactobacillus* strains in environments inhabited by pathogenic microorganisms contributes to ecological balance. These probiotic bacteria compete with pathogens for nutrients and physical space, thereby limiting the proliferation of opportunistic species such as *E. coli*. Cell-free supernatants (CFSs) derived from all tested *Lactobacillus* strains demonstrated antimicrobial activity, showing moderate to strong inhibitory effects against *B. cereus*, a common cause of foodborne illness. *B. cereus* is frequently isolated from improperly cooked or stored food products, including vegetables and dairy-based items. Numerous studies have confirmed the high virulence of *B. cereus*, which is responsible for a significant proportion of food poisoning-related fatalities (39). The application of *Lactobacillus*-derived CFSs as natural antimicrobial agents presents a promising strategy for food preservation and the control of foodborne pathogens, particularly those that are multidrug-resistant (MDR). This approach aligns with growing interest in developing safe, effective, and natural alternatives to synthetic preservatives in the food industry.

Conclusions

Lactobacillus strains can inhibit multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria by secreting antigens, proteins, and acidic compounds that disrupt the adhesion processes of pathogenic bacteria to the host's living tissues and reduce the formation of anti-inflammatory agents, such as interleukins. Additionally, they have the ability, through specific mechanisms, to denature the amino acids of pathogenic bacteria and limit their spread. *Lactobacillus* strains maintain the environmental balance of pathogenic bacteria in the environment. *Lactobacillus* strains can inhibit Gram-negative bacteria more effectively than they can inhibit Gram-positive bacteria.

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المعززات الحيوية وتأثيرها على البكتيريا الموجبة وسالبة لصبغة الغرام الممرضة

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الخلاصة

خلفية عن الموضوع: أن هناك اكثر من اربعة سلالات من بكتيريا العصيات اللبنية، لديها القدرة على تثبيط وقتل البكتيريا المقاومة للمضادات الحيوية، وخاصة المكورات العنقودية الذهبية والزائفة الزنجارية. وذلك لامتلاكها آليات محددة مثل إنتاج وتكوين الإنزيمات والأسيتالديهايد والأحماض العضوية والبكتريوسينات وبيروكسيد الهيدروجين وغيرها، بالإضافة إلى تكوين حمض اللاكتيك. يتم تشخيص البكتيريا المسببة للأمراض من خلال خصائصها الشكلية على أوساط الزراعة التفاضلية، وقدرتها على تخمير اللاكتوز، وإنتاج المخاط والهيموليسين. يتم تشخيص العزلات عن طريق الاختبارات الكيميائية الحيوية بثلاثة اختبارات رئيسية وهي إنتاج إنزيم الكاتالاز، واختبار تخمر أوكسيولاز، واختبار التخثر، بينما يتم التشخيص الجزيئي باستخدام اختبارات PCR وتقنية VITEK2. **النتائج:** أثبتت الدراسة أن البكتيريا المقاومة للمضادات الحيوية لديها القدرة على إنتاج جينات الفوعة مثل *coa* و*nuc* و*fimH* و*hlg* و*hla*، والتي تنتشر في بكتريا المكورات العنقودية الذهبية، بينا تعود جينات *hlyA* و*fimH* إلى البكتيريا سالبة الجرام. أن بكتيريا *Lactobacillus* يمكنها الارتباط بخلايا الأنسجة الحية للمضيف عن طريق بروتينات الالتصاق وتمنع بكتيريا *S. aureus* من إصابة أنسجة المضيف بنسبة تصل الى 80%، وبالتالي الحفاظ على التوازن البيئي والحياتي داخل المضيف والحد من تكوين السيتوكينات المضادة للالتهابات مثل الإنترلوكين وخاصة (IL17). كما وجد ايضا أن *Lactobacillus* بشكل عام لديها القدرة على تثبيط وقتل البكتيريا سالبة الجرام المقاومة للمضادات الحيوية (MDA). **الاستنتاج:** يمكن أن تمنع سلالات *Lactobacillus* البكتيريا الموجبة والسالبة لصبغة الغرام ذات المقاومة المتعددة للمضادات الحيوية (MDR) من الانتشار والتكاثر عن طريق إفراز المستضدات والبروتينات والمركبات الحمضية التي تعطل عمليات التصاق البكتيريا المسببة للأمراض بالأنسجة الحية للمضيف.

الكلمات المفتاحية: المقاومة المتعددة للمضادات الحيوية، جينات الضراوة، *Lactobacillus*، بكتريا موجبة لصبغة الغرام، سالبة لصبغة الغرام .