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Antibacterial Activity of *Lactobacillus reuteri*–Mediated Biosynthesized Silver Nanoparticles Against Multidrug-Resistant Bacterial Isolates from Burn and Ulcer Wounds: An In Vitro Study

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Abstract

Background: Burns and ulcerative wounds are high-risk sites for multidrug-resistant bacterial infections. Silver nanoparticles synthesized using Lactobacillus reuteri bacteria may offer an environmentally friendly and sustainable alternative with potent antibacterial activity. **Objective: "**This study represents a scientific effort to evaluate the inhibitory efficacy of silver nanoparticles **(AgNPs)** synthesized via a green pathway, using *Lactobacillus reuteri* as both reducing agent and biological carrier, against clinically isolated antibiotic-resistant bacteria from burn and ulcer wounds. **Methodology:** Isolates were obtained from patients at Al-Hussein Medical City's hospital laboratory in Karbala, Iraq, during October 2024 to March 2025. The study was grounded in the hypothesis that integrating *Lactobacillus*'s biological properties—as part of normal flora, producing organic acids and bacteriocins—with silver nanoparticles' unique physicochemical traits would yield a synergistic effect capable of overcoming complex bacterial resistance mechanisms.". The practical aspect of the research involved collecting twenty clinical swabs from infection sites and transferring them to sterile media to ensure accurate retrieval of bacterial isolates. Critical bacterial strains were identified, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter*. Their identity and resistance patterns were confirmed through a series of biochemical and susceptibility tests according to the Clinical Laboratory Standards Institute (CLSI) criteria. This was followed by the biosynthesis of nanoparticles, utilizing the reducing capacity of naturally occurring *Lactobacillus*

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reuteri to convert silver ions into nanoparticles. These nanoparticles were then characterized using UV-Vis spectroscopy to determine the absorption peak and electron microscopy to verify the size distribution and morphology of the resulting particles. **Results:** The expression levels of resistance genes were assessed, and silver nanoparticles (AgNPs) were found to inhibit the growth of all tested multidrug-resistant (MDR) bacterial strains. The diameters of the inhibition zones (MZs) (mm) at concentrations of 15, 20, and 25 µg/ml were as follows: Staphylococcus aureus 24.0–27.0, vancomycin-resistant *Enterococcus* 21.0–26.0, *Acinetobacter baumannii* 19.0–23.0, *Pseudomonas aeruginosa* 17.0–22; *Klebsiella pneumoniae* 16–18 mm; *Escherichia coli* 13–17 mm. The minimum inhibitory concentration (MIC) ranged between 15 and 25 µg/ml. Gram-positive bacteria were more susceptible than Gram-negative bacteria. The observed differences were statistically significant between species and concentrations ($p<0.05$). Results: Traditional antibiotics showed varying sensitivity, with some Armenian Protestant strains showing resistance to all classes of drugs tested. **Conclusions:** This proves that this green nanotechnology approach represents a promising and low-cost strategic alternative to address the escalating microbial resistance crisis in clinical settings.

Keywords: Antibiotics, Multiple drug resistance (MDR), Burn injuries Synergistic effect, *Lactobacillus reutter*, green synthesis, silver nanoparticles (AgNPs), Ulcerative wounds

Introduction

Antibiotic resistance poses a growing threat to global public health, particularly in hospital settings with high rates of wound and burn infections. The overuse and uncontrolled use of broad-spectrum antibiotics have accelerated the emergence of multidrug-resistant (MDR) bacteria, most notably the group known as ESKAPE pathogens. The danger of this group lies in its remarkable ability to evade conventional drug-killing mechanisms through complex defense strategies, including: changing the antibiotic target site, activating efflux pumps to expel the drug, and enzymatic degradation of drug compounds before they reach their targets. These mechanisms perpetuate infections, leading to prolonged hospital stays, increased economic burdens, and higher morbidity and mortality rates, especially among patients with chronic ulcers and severe burns [1][2].

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Burns and ulcerative wounds present an ideal environment for microbial growth. Factors such as high bacterial load, weakened host immune response, and the micro environmental conditions of the wound combine to exacerbate the infection. These infections are often associated with Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and Gram-positive bacteria such as *Staphylococcus aureus*. These pathogens are characterized by their high capacity for biofilm formation cellular matrices that protect bacteria and impede antibiotic penetration, thus promoting their survival even under harsh conditions and rendering conventional treatments ineffective. This reality underscores the urgent need to develop innovative therapeutic strategies that transcend the limitations of traditional mechanisms [3][4].

Nanotechnology has emerged as a promising solution to overcome the limitations of antibiotics, with silver nanoparticles (AgNPs) demonstrating remarkable efficacy as broad-spectrum antimicrobial agents. This is due to their physical stability and unique ability to target microbes through multiple, simultaneous mechanisms, including inducing oxidative stress (ROS) generation, causing direct DNA damage, and destabilizing the cell membrane. This multifaceted offensive nature significantly reduces the likelihood of bacteria developing resistance, making silver nanoparticles a strong candidate for combating pathogens associated with burn and wound injuries [5]; [6][7]. The biosynthesis of nanoparticles, known as "green synthesis," has gained considerable scientific traction as an environmentally safe and economical alternative to chemical and physical methods. This technique utilizes beneficial microorganisms, such as *Lactobacillus reuteri*, as natural reducing and stabilizing agents, eliminating the need for toxic solvents and reducing energy consumption. Biosynthesized silver nanoparticles are characterized by high biocompatibility and enhanced stability, as well as superior antimicrobial activity, making them suitable for advanced medical applications including wound sterilization and the control of intractable infections [8][9].

Clinically, studies have demonstrated the efficacy of silver nanoparticles in penetrating bacterial biofilms and inhibiting bacterial growth while preserving the integrity of human tissues. This promotes their use in dressings and topical gels as part of wound management programs [10][11]. In line with the World Health Organization's (WHO) call for innovative solutions, this study evaluates the inhibitory activity of

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biosynthesized silver nanoparticles using *L. reuteri* against resistant bacterial isolates obtained from wound and burn patients at Al-Husseini Hospital in Karbala Governorate. The study aims to provide scientific evidence supporting green nanotechnology as an effective and sustainable therapeutic approach in the Iraqi clinical setting

Study Objective:

This study primarily aims to evaluate the inhibitory efficacy of biosynthesized silver nanoparticles (AgNPs) using *Lactobacillus reuteri* bacteria against multidrug-resistant (MDR) bacterial strains isolated from ulcerative wounds and burns at Al-Husseini Hospital in Karbala Governorate. The study also seeks to compare the efficacy of these bioparticles with conventional antibiotics to determine their suitability as a therapeutic alternative or synergistic agent in current treatment protocols.

Materials and Methods

Preparation and Green Biosynthesis of Silver Nanoparticles (AgNPs) : The environmentally friendly biosynthesis method was employed to synthesize silver nanoparticles using Lactobacillus isolates, as a safe alternative to chemical and physical methods, based on standard protocols in this field [12].

1- Preparation of Bacterial Biomass: To obtain the bioreducing agents, *Lactobacillus reuteri* was cultured on MRS agar under standard incubation conditions. The bacterial colonies were then extracted and transferred to deionized water to produce a biomass suspension containing the enzymes and proteins responsible for the reduction process [13].

2- Precursor Preparation: A silver nitrate ($AgNO_3$) solution was prepared at a precise concentration of 1 mM. To ensure the stability of the solution and prevent spontaneous photoreduction, it was stored in opaque containers away from direct light, according to the recommendations of [14].

3- Synthesis Procedure: The synthesis was carried out by gradually adding 10 mL of the bacterial suspension (drop by drop) to 90 mL of the silver nitrate solution under continuous stirring at 35 °C. The color change of the mixture from pale yellow to reddish-brown was observed, which is the primary physical indicator of surface

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plasmon resonance and nanoparticle formation**.** This change is a key physical indicator of surface plasmon resonance and nanoparticle formation [15].

4- Structural and Morphological Characterization Techniques: To confirm the physical and chemical properties of the synthesized particles, the following tests were performed**:**

a. UV-Vis Spectroscopy: To determine the surface absorption peak within the 200-800 nm wavelength range.
b. Scanning Electron Microscopy (SEM): To study the surface morphology and particle size distribution.
c. X-ray Diffraction (XRD): To determine the crystalline phase and the material's purity.
d. Atomic Force Microscopy (AFM): To obtain three-dimensional images and measure surface roughness at nanometer resolution.

5. The effectiveness of the prepared nanocomposite (*Lactobacillus Spp*. + silver nanoparticles) in inhibiting the growth of resistant bacteria was evaluated using two in vitro methods to ensure the reliability of the results: Agar-well diffusion method: This method is a standard approach for assessing the sensitivity of microorganisms to extracts and nanocomposites. It was applied according to the methodology of [16] with some modifications; Medium preparation and inoculation: Nutrient agar was poured into sterile Petri dishes. After solidification, the bacterial isolate was evenly distributed on the surface of the medium using a sterile cotton swab to ensure uniform growth. Using a sterile cork drill of a specified diameter, regular wells were made in the agar layer. Graduated volumes (15, 20, and 25) µL of the silver-loaded *Lactobacillus* complex were added to the wells. The dishes were incubated at 37°C for 24–48 hours. Effectiveness was determined by measuring the diameters of the inhibition zones (in millimeters) around each well [17].

Toxic Food Technique: This technique is used to evaluate the effectiveness of a substance when mixed directly with a nutrient medium. The methodology described by [18] was followed: Preparation of the Toxic Medium: A silver nanoparticle/liquid lactobacillus (AgNPs) complex was added to sterile agar medium cooled to approximately 45°C (before solidification) at a ratio of 20 mL of the complex to 200 mL of the nutrient medium. The mixed medium was poured into dishes, and after

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complete solidification, resistant bacteria were inoculated into the center of the dish. For the control group, dishes containing a nutrient medium without the nanoparticle complex were used for standard comparison. After the incubation period, the growth of bacteria in the poisoned dishes was compared with the control group, and the percentage of growth Inhibition Zones was calculated according to the standard equation of [19].

Antibiotic Susceptibility Testing: The resistance pattern determination phase is a pivotal step in studying bacterial isolates and was performed according to the following methodology:

1. Methodology and Standards: Antibiotic resistance patterns were assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. All tests and interpretation of results were carried out strictly according to the recommendations and guidelines of the Clinical and Laboratory Standards Institute [20]

2. List of Antibiotics Tested: The study included a wide range of antibiotics (19 antibiotics) to ensure coverage of various drug classes, namely:

Group 1: AK, AZM, AMP, AUG, CRO, CTX

Group 2: PIP, SXT, MEM, CAZ, CFM, CIP

Group 3: CHL, FEP, GEN, LVX, NAL, NIT, TCY 3. Multidrug Resistance (MDR) Classification. The international standard for classifying isolates as multidrug resistant (MDR) is adopted if a bacterial isolate exhibits resistance to one or more drugs in three or more biological classes.

Statistical Analysis:

All experiments were performed in triplicate. Data were analyzed using SPSS software. Comparisons between bacterial responses to AgNPs and conventional antibiotics were performed using t-tests or one-way ANOVA. A p-value of less than 0.05 is considered statistically significant. [21]

Ethical approval: Ethical approval was obtained from the Institutional Review Board of Al Hussein Medical City, Karbala, Iraq 2024, and patient consent was waived for the remaining clinical specimens.

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Results and Discussion

The results shown in Table (1) of the Kirby-Bauer method for antibiotic susceptibility testing reveal a marked variability in the bacterial isolate's response to different antibiotic groups. The bacteria exhibited absolute resistance to oxacillin (inhibition diameter 0 mm). This resistance in Gram-positive strains, specifically the *Staphylococcaceae* family, is attributed to the presence of the mecA gene, which alters the denaturation of penicillin-binding proteins (PBP2a), thus preventing beta-lactam antibiotics from binding to the cell wall [20]. In contrast, the fluoroquinolone group (ciprofloxacin and levofloxacin) showed high inhibition values (23.3, 19) mm, respectively. This in vitro superiority is attributed to the high ability of these compounds to penetrate the cell membrane and inhibit DNA gyrase and topoisomerase IV enzymes, leading to the cessation of bacterial DNA replication and cell death. The similarity in results between these two antibiotics indicates the sensitivity of the target site in this strain to this chemical family [22].

Table (1) Antibiotic susceptibility testing reveal a marked variability in the bacterial *Staphylococcus aureus*

Antibiotic Cod	Antibiotic (Symbol) Scientific Name of Antibacterial	Inhibition Zone (mm) of Antibacterial	Phenotypic State
F 300	Nitrofurantoin	26	Sensitive
CIP 5	Ciprofloxacin	23.3	Sensitive
LEV 5	Levofloxacin	19	Sensitive
E 15	Erythromycin	18	Sensitive
TE 30	Tetracycline	18.3	Sensitive
AZM 15	Azithromycin	15.3	Sensitive
OX 1	0	0	Resistant
Control	0	0	
2.18			LSD value P≤0.05

On the other hand, the results indicated an intermediate response to erythromycin and tetracycline, suggesting the presence of primary resistance mechanisms, possibly via efflux pumps that reduce the intracellular concentration of the antibiotic before it reaches the 30S or 50S ribosomal subunits. The smaller diameter of azithromycin (15.3 mm) compared to the others suggests a partial mutation in the RNA that reduces its

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binding affinity [23]. Figure (1). Furthermore, nitrofurantoin exhibited the highest inhibition value (26 mm), a highly statistically significant result. This is explained by its multiple mechanisms of action; it is reduced within bacteria to highly reactive intermediates that simultaneously attack proteins, ribosomes, and DNA, making the development of resistance against it difficult and complex for bacteria in the short term [24][25]. This confirms the LSD value of 2.18, indicating significant differences among most of the tested antibiotics. From a research perspective, this reinforces the hypothesis that the selection of the appropriate antibiotic should be based on laboratory results rather than clinical guesswork, as the differences in inhibition diameters exceeded the experimental error threshold.

Figure (1) shows *Staphylococcus aureus* bacteria in the Kirby-Bauer antibiotic susceptibility test.

On the other hand, the statistical analysis results in Table (2) indicate the sensitivity of *E. coli* to antibiotics, as they cause infections of surgical and purulent wounds, post-operative wounds, and deep skin infections. The results showed that *E. coli* was "very sensitive" or "sensitive" to a group of antibiotics, which are the best for use in treatment: Quinolones: These achieved the highest inhibition rates. Levofloxacin (LEV 15 & LEV 5) showed very high inhibition diameters (more than 25 mm), indicating the superior efficacy of this antibiotic against the tested strain. Ciprofloxacin (CIP 5) showed an excellent response (23 mm). These antibiotics work by inhibiting the DNA gyrase enzyme, which is essential for DNA replication in *E. coli*. Tetracycline (TE 30): The result (19 mm) falls within the sensitive range. This antibody works by inhibiting protein synthesis through binding to the 30S ribosomal subunit, while gentamicin (CN

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10) showed good sensitivity (16 mm). It is an aminoglycoside that causes misreading of bacterial mRNA [26][27].

Table (2): Antibiotic susceptibility testing reveal a marked variability in the bacterial *E. coli*.

Antibiotic Cod	Antibiotic (Symbol) Scientific Name of Antibacterial	Inhibition Zone (mm) of Antibacterial	Phenotypic State
LEV 15	Levofloxacin	25.33	Sensitive
CIP 5	Ciprofloxacin	23.0	Sensitive
LEV 5	Levofloxacin	26.33	Sensitive
TE 30	Tetracycline	19	Sensitive
CN 10	Gentamicin	16	Sensitive
AZM 15	Azithromycin	7,67	Resistant
E 15	Erythromycin	0	Resistant
OX 1	Oxacillin	0	Resistant
Control		0	
1.51			LSD value P≤0.05

Furthermore, the strain showed clear resistance to three types of antibiotics: Oxacillin (OX1) and erythromycin (E15) showed an inhibition diameter of 0 mm, which is absolute resistance. This is normal for *E. coli* (a Gram-negative bacterium) to be resistant to oxacillin because its cell wall contains an outer membrane that prevents the penetration of antibiotics intended for Gram-positive bacteria. Azithromycin (AZM15), however, showed a very weak inhibition diameter (7.67 mm), classifying it as a resistant bacterium.

The phenomenon of multidrug resistance (MDR) is known in *E. coli*, which have shown resistance to different classes (macrolides and penicillins). This suggests the possibility that the strain possesses resistance mechanisms such as efflux pumps or enzymes. Beta-lactamase. Additionally, it possesses the mecA gene, which alters the shape of cell wall proteins, rendering the entire penicillin and cephalosporin family ineffective. Overlapping phenomenon: We observe at the bottom of the plate that the inhibition zones of LEV 15, CIP 5, and LEV 5 have fused together. This indicates a very high sensitivity of this class of antibiotics (fluoroquinolones) to this bacterium.

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Furthermore, the bacterium exhibits clear resistance to oxacillin (OX 1) and erythromycin (E 15), and the inhibition zone around azithromycin (AZM 15) is very narrow, suggesting resistance as well. Figure (2)

On the other hand, the results in Table (3) and figure (3) indicate the multiple resistance mechanisms possessed by *Pseudomonas aeruginosa*, a Gram-negative bacterium classified by the (WHO) as one of the most dangerous antibiotic-resistant bacteria (MDRs). The results show complete resistance to antibiotics such as Cefotaxime (CTX), Azithromycin (AZM), and Nalidixic Acid (NA). This is attributed to *P. aeruginosa* possessing very low outer membrane permeability, in addition to the presence of active efflux pumps that expel the antibiotic before it reaches its target within the cell.

Table (3) Antibiotic susceptibility testing reveal a marked variability in the *Pseudomonas aeruginosa*

Antibiotic Cod	Antibiotic (Symbol) Scientific Name of Antibacterial	Inhibition Zone (mm) of Antibacterial	Phenotypic State
LEV 5	Levofloxacin	14,67	Sensitive
TE 30	Tetracycline	13	Sensitive
MRP 10	Meropenem	0	Resistant
CTX 30	Cefotaxime	0	Resistant
AK 30	Amikacin	0	Resistant
CIP 5	Ciprofloxacin	0	Resistant
CN 10	Gentamicin	0	Resistant
NA 30	Nalidixic Acid	0	Resistant
AZM 15	Azithromycin	0	Resistant
Control		0	
0,62			LSD value P≤0.05

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Furthermore, resistance to meropenem (MRP) was shown, with 0 mM being the most severe resistance identified in the table. Meropenem is a carbapenem antibiotic, and this resistance often results from the loss of the OprD channel protein (which allows carbapenem entry) or the production of carbapenemases that completely degrade the drug. Resistance to aminoglycosides (amikacin and gentamicin) was 0 mM for both AK and CN. This is usually due to aminoglycoside modification enzymes (AMEs) that alter the antibiotic's structure, or by modifying messenger RNA (mRNA) molecules (16S rRNA methyltransferases) that prevent the antibiotic from binding to bacterial ribosomes. The limited sensitivity to levofloxacin (LEV) resulted in a halo (14.67 mm), indicating minimal susceptibility. This is attributed to resistance in the quinolone family, which arises from mutations in the gyrA or parC genes that alter the shape of the target enzymes (DNA gyrases), thus impairing drug binding. A value of 14 mm often falls within the "resistant" range according to CLSI criteria. These bacteria employ a defensive "arsenal" that includes: efflux pumps, such as the MexAB-OprM system, which expel quinolones and beta-lactams; beta-lactamases, such as AmpC, which degrade cephalosporins; and altered membrane permeability, reducing the number of pores in the outer membrane. (Microbiology and Infection. [24] [28][29].

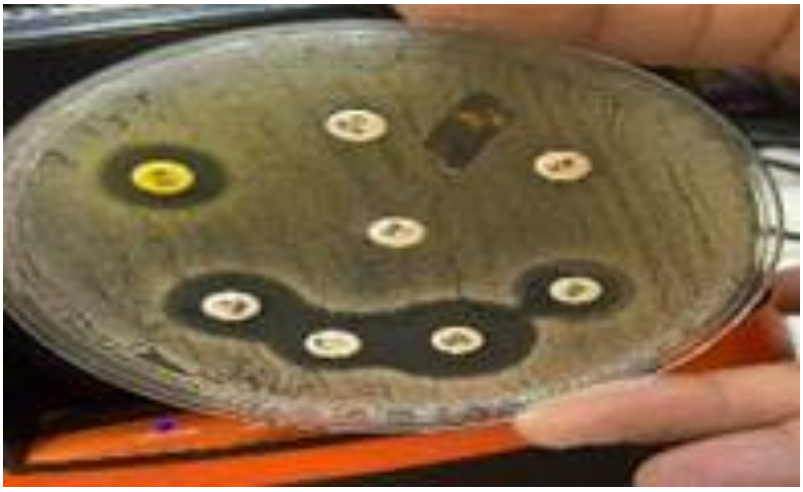

Figure (2) *Pseudomonas aeruginosa*, multidrug-resistant (MDR), is considered the number one enemy of burn patients.

On the other hand, Table (4) shows the response of *E. coli* to nine different antibiotics, measured by the diameter of the inhibition zone in millimeters (mm). Carbapenems were the most effective. Imipenem (IMI) and meropenem (MRP) showed the highest inhibition rates (30.67, 28) mm, respectively. These antibiotics are considered "last resort" against enteric bacteria. They work by binding to penicillin-

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binding proteins (PBPs), leading to bacterial cell wall lysis. They exhibit high resistance to beta-lactamase enzymes secreted by some *E. coli* strains. Aminoglycosides also showed excellent efficacy. Amikacin (AK) and gentamicin (CN) demonstrated excellent results (25.67 mm and 21 mm, respectively). These antibiotics work by inhibiting protein synthesis within the bacterial cell through binding to the 30S ribosomal subunit. The continued sensitivity of the bacteria to this group of drugs indicates the absence of mutations in ribosomes or efflux pumps that would activate these drugs. While the results showed sensitivity to fluoroquinolones, ciprofloxacin (CIP) and levofloxacin (LEV) exhibited large inhibition zones (approximately 25 mm). These drugs target the DNA gyrase enzyme responsible for DNA unwinding. The results suggest that the tested strain does not possess mutations in the gyrA or parC genes. Figure (3).

Table (4) *E. coli* produces enzymes that break down the penicillin and cephalosporin families. The plate is distinctive because it shows very high bacterial sensitivity to all tested antibiotics.

Antibiotic Cod	Antibiotic (Symbol) Scientific Name of Antibacterial	Inhibition Zone (mm) of Antibacterial	Phenotypic State
IMI 10	Imipenem	30.67	Sensitive
MRP 10	Meropenem	28	Sensitive
CTX 30	Cefotaxime	26	Sensitive
AK 30	Amikacin	25.67	Sensitive
CIP 5	Ciprofloxacin	25	Sensitive
LEV 5	Levofloxacin	24.67	Sensitive
SXT 25	Sulfamethoxazole	24	Sensitive
CN 10	Gentamicin	21	Sensitive
NA 30	Nalidixic Acid	20.33	Sensitive
Control		0	
1.16			LSD value P≤0.05

The results shown in the table indicate that the *E. coli* strain used in the experiment is a highly susceptible (wild-type or non-MDR) strain, as it did not exhibit resistance to any of the tested antibiotics, including nalidixic acid (NA), which represents the first generation of quinolones. [20][30][31].

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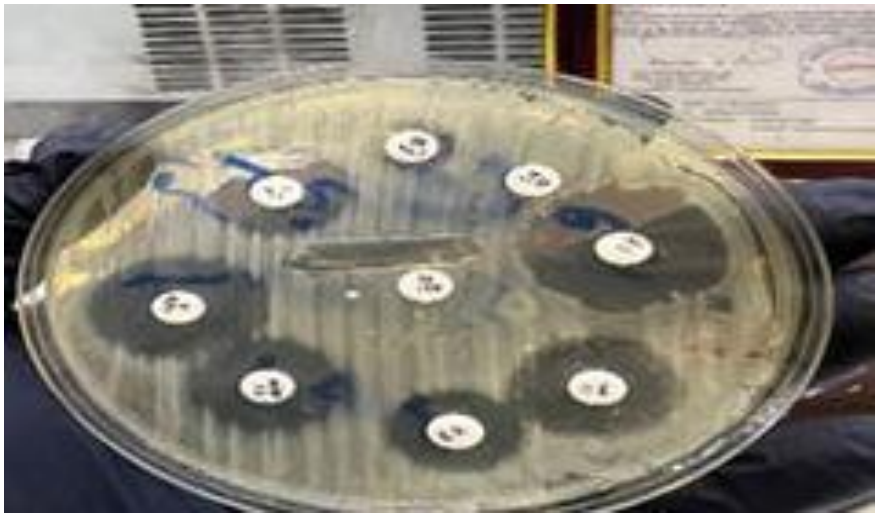

Figure (3) *E. coli* produces enzymes that break down the penicillin and cephalosporin families.

On the other hand, the results indicated the sensitivity of *Acinetobacter baumannii* to the antibiotics listed in Table (5), showing high resistance, a type typically associated with "superbugs." Phenotypic profiling revealed that the strain exhibits multidrug resistance (MDR), demonstrating complete resistance to at least three drug classes: fluoroquinolones, cephalosporins, and partial carbapenems. The carbapenem paradox is also noted, where the bacteria exhibit sensitivity to imipenem and complete resistance to meropenem. Resistance in A. baumannii often depends on the loss of outer membrane proteins (porins), such as CarO. Studies have shown that loss of the CarO protein selectively affects meropenem permeability more than imipenem, which explains this discrepancy. The overall resistance to cephalosporins, specifically to ceftazidime and cefepime (0 mM), clearly indicates that the bacteria produce AmpC beta-lactamases or OXA-type carbapenemases (such as OXA-23 or OXA-51) that are genetically induced (insertion sequences such as ISAba1).

Table (5) Antibiotic susceptibility testing reveal a marked variability in the *Acinetobacter baumannii*

Antibiotic Cod	Antibiotic (Symbol) Scientific Name of Antibacterial	Inhibition Zone (mm) of Antibacterial	Phenotypic State
AK 30	Amikacin	29	Sensitive
IMI 10	Imipenem	24.33	Sensitive

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TOB 30	Tobramycin	21	Sensitive
CN 10	Gentamicin	12.67	Sensitive
MRP 10	Meropenem	0	Resistant
CIP 5	Ciprofloxacin	0	Resistant
LEV 5	Levofloxacin	0	Resistant
CAZ 30	Ceftazidime	0	Resistant
FEP 30	Cefepime	0	Resistant
Control		0	
1.67			LSD value P≤0.05

Regarding aminoglycoside efficacy, the high sensitivity of amikacin (29 mM) and tobramycin (21 mM) is a turning point in this assay figure (5). The absence of aminoglycoside-modifying enzymes (AMEs) in this strain makes these drugs the optimal treatment option. However, their use as monotherapy in cases of bloodstream infections is strongly discouraged to prevent the rapid development of resistance. On the other hand, fluoroquinolone resistance, including resistance to ciprofloxacin and levofloxacin (0 mM), reflects mutations in the genes encoding target enzymes: gyrA: responsible for DNA gyrase. parC: responsible for topoisomerase IV. These mutations are very common in nosocomial strains. The resistance pattern also shows that bacteria exhibit complete resistance to a wide range of antibiotics, including quinolones (CIP, LEV), cephalosporins (CAZ, FEP), and meropenem (MRP). Interestingly, the bacteria are sensitive to imipenem (IMI) but resistant to meropenem (MRP).

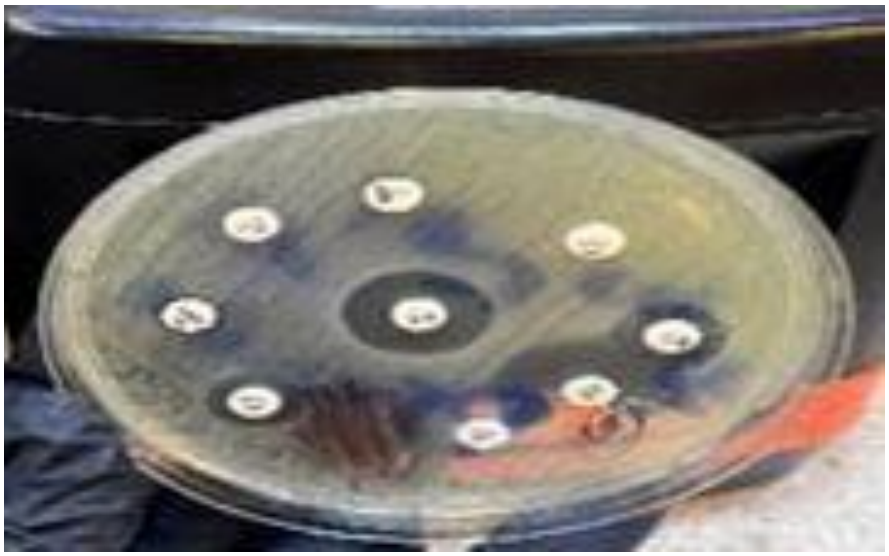

Figure (4) ***Acinetobacter baumannii*****, multidrug-resistant (MDR), is considered the number one enemy of burn patients.**

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It was also observed in the table that the results regarding the sensitivity of traditional antibiotics are sometimes sensitive in dishes, but the same antibiotics fail in wounds and burns, especially in hospitalized patients. This can be explained as follows: Either due to poor blood supply, especially in severe burns caused by the destruction of blood vessels, the antibiotic the patient is taking (orally or intravenously) does not reach the site of the bacteria in the wound in sufficient quantities [32][33]. Also, the presence of multiple strains at the same site of a wound or burn can lead to the simultaneous presence of aerobic and anaerobic bacteria, and even other pathogens, in a single wound. This necessitates a combination of antibiotics. Furthermore, bacteria within the wound develop acquired resistance, rapidly exchanging resistance genes [34]. This is precisely what we see in the differences between the petri dishes, as shown in the previously presented images. This source explains why antibiotics kill bacteria in petri dishes but fail in wounds; the reason is the "biofilm" of the wound, which protects it and allows for gene exchange. In petri dishes, bacteria are planktonic and easily killed, while in wounds, the types mentioned are among the most dangerous bacterial strains in hospitals because they have developed complex mechanisms to resist treatment. [35][36][37]. Second, the green nanoparticle antibody test involved two methods:

a. Biosynthesized silver nanoparticles demonstrated potent inhibitory activity against multidrug-resistant bacteria isolated from wound and burn infections.
b. Particularly susceptible isolates included MRSA, VRE, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli*, and other pathogens resistant to conventional antibiotics.
c. The results suggest that these particles represent a promising alternative or effective complement in clinical settings with high resistance to conventional antibiotics. The study underscores the importance of green nanotechnology in developing effective and environmentally sustainable antimicrobial strategies, potentially offering an additional immunological advantage through the use of *Lactobacillus reuteri,* which is part of the natural human microbiota. The results in Table (6) for the variance analysis between Gram-positive and Gram-negative bacteria show that the highest inhibition values were for *Staphylococcus* and VRE, both Gram-positive bacteria. This is attributed to these bacteria possessing a cell wall composed of a thick peptidoglycan layer containing pores that allow nanoparticles to pass more easily into the cytoplasmic membrane. In contrast, for Gram-negative bacteria

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(*Pseudomonas* and *Klebsiella*), we observe a decrease in inhibition diameters. The reason is that it has an outer membrane rich in glycolipids (LPS), which acts as an additional selective barrier that makes it difficult for nanoparticles to penetrate, which explains the need for higher concentrations (such as 25 mM) to achieve significant inhibition.

Increasing the concentration from 15 to 25 mM not only increased the number of particles, but also increased the flux density on the surface of the bacterial cell: at a concentration of 15 mM, the particles may be satisfied with causing a state of "oxidative stress". At a concentration of 25 mM, the nano-pressure exceeds the bacteria's repair capacity, leading to DNA fragmentation and inhibition of vital enzyme activity. This explains the diameter increase from 24 mm to 27 mm in *Staphylococcus* [38]. Furthermore, the results of the LSD analysis ($p \leq 0.05$) are statistically significant. An LSD value of 1.86 indicates that any difference between the two means in the table exceeding this value is a genuine difference resulting from the nano-treatment and not merely an experimental error. The difference between the effects of concentrations 15 and 25 mM in Acinetobacter bacteria is 23 - 19 = 4 mM. Since 4 mM > 1.86, the nanomaterial is "substantially" effective in eliminating this resistant bacterium at higher doses. This is due to the "green nanoparticle" (Green Synthesis). The "deep" power of the green method lies in a phenomenon called the synergistic effect. Chemically synthesized nanoparticles are "bare," while green nanoparticles are coated with phytochemicals from plant extracts (such as phenols and flavonoids). These compounds act in a deceptive manner, tricking the bacterial cell and helping the nanoparticles adhere to the membrane. The nanoparticles inside then release metal ions that are toxic to the cell.

Table (6) Biosynthesized silver nanoparticles demonstrated potent inhibitory activity against multidrug-resistant bacteria isolated from wound and burn infections.

Resistant bacteria type	The bacterial rate	Diameter of inhibition Zone (mm)*			Sensitivity level
		25 mM	20 mM	15 mM	
Staphylococcus aureus	25.67	27	26	24	High
VRE (vancomycin-resistant enterococci)	23.33	26	23	21	High
Acinetobacter baumannii	21	23	21	19	High

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Pseudomonas aeruginosa	19.33	22	19	17	Moderate
Klebsiella pneumonia	16.33	13	18	16	Moderate
resistant bacteria type	0	0	0	0	Resistant
Concentration rate	17.61	18.38	17.83	16.17	
LSD value conc. 1.86 bacteria 2.63 P≤0.05					

Figure (5) Biosynthesized silver nanoparticles demonstrated potent inhibitory activity against multidrug-resistant bacteria isolated from wound and burn infections.

The results shown in the table are consistent with the study by Marimuthu et al., (2020), which confirmed the effectiveness of nanoparticles against VRE bacteria, where high concentrations (25 mM) demonstrated a superior inhibition capacity of 26 mM. The difference in sensitivity between *Staphylococcus* and *Klebsiella* is also

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attributed to the physical mechanisms described by [39], where surface charge and particle size play a pivotal role in penetrating different cell walls, as confirmed by the LSD values of 1.86, which prove the significance of the differences between the concentrations used.

On the other hand, the results showed excellent inhibitory activity of the *L. reuteri*-AgNPs compound against all tested resistant isolates. Staphylococcus bacteria exhibited the highest sensitivity to the compound, followed by *Pseudomonas aeruginosa and Klebsiella pneumoniae.*This strong effect is attributed to a synergistic effect, whereby *Lactobacillus* biosecretion lowers the pH and increases cell membrane permeability, thus paving the way for silver nanoparticles to penetrate the cell and disrupt its genetic content. Furthermore, the etching method provided a clear indication of the material's ability to diffuse and kill bacteria even at dilute concentrations reaching the periphery of the halo.

A. Well Diffusion Method: This method measures the diameter of the inhibition zone. The larger the diameter of the transparent zone around the well, the greater the ability of the compound (*Lactobacillus* + silver) to diffuse in the culture medium and kill bacteria at a specific concentration. Table (6) Interpretation of Irregular Zone Shapes: Since the material was injected as a liquid into the wells, this explains the irregular shape of the zones (Irregular Inhibition Zones). Scientifically, this does not diminish the value of the result and can be explained as follows: The diffusion rate of nanomaterials loaded onto biological media (such as *Lactobacillus*) may not diffuse perfectly evenly in the agar due to the viscosity of the medium or the interaction between the molecules. Additionally, hydraulic pressure: When the liquid is injected into the well, some of it may leak under or over the agar layer if the well is not perfectly sealed, changing the zone shape from a regular circle to an oval or zigzag shape.

During measurement, the average diameter of the two auras (long and short) was taken to obtain a precise figure that approximates the effectiveness. The results shown in Table (7) indicate that the silver nanoparticles, or "nanoparticles," produced by Lactobacillus bacteria, possess very high inhibitory activity against a range of pathogenic bacteria, particularly *Staphylococcus aureus* and antibiotic-resistant bacteria. Inhibition rates at a concentration of 20 mM ranged from (78, 95) %, which is a very strong indicator of the efficiency of the nanomaterial used. The nanoparticles

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(prepared by *Lactobacillus*) adhere to and disrupt the bacterial cell wall, leading to leakage of cellular contents. They also release metal ions that stimulate the formation of free radicals (ROS), which damage the DNA and proteins within the bacteria. Furthermore, the Lactobacillus nanomaterial's biosynthetic nature gives it the advantage of being "biosynthesized." The enzymes secreted by these bacteria (such as reductases) act as reducing agents and capping agents, making the nanoparticles more stable and less toxic to humans compared to chemical methods.

On the other hand, the manufactured nanomaterial demonstrates its ability to inhibit resistant bacteria (VRE and *Staphylococcus*). Studies indicate that the nanoparticles evade traditional resistance mechanisms (such as centrifugal pumps or antibiotic-degrading enzymes) because their small size allows them to penetrate the cell directly. Furthermore, despite their thickness, their cell walls lack the outer lipid membrane found in Gram-negative bacteria, facilitating the entry of silver nanoparticles into the cytoplasm. The control treatment was resistant, proving that the inhibition is indeed due to the nanomaterial and not the solvent or experimental conditions. Gram-negative bacteria, specifically *Pseudomonas and Klebsiella*, showed an excellent response (17-19 mm). The organic acids produced by *Lactobacillus* acted as a "key" to open gaps in the outer membrane, enabling the silver nanoparticles to enter. The inhibitory power of the silver nanoparticle solution not only prevents diffusion but also renders the entire culture medium unsuitable for the growth of resistant bacteria. [40][41][42].

The results obtained from both methods (pit diffusion and poisoned food) show that the silver-based nanocomposite derived from *Lactobacillus reteus* and loaded with silver nanoparticles possesses broad-spectrum antimicrobial properties, particularly against highly resistant bacterial strains (ESKAPE). As demonstrated by comparing the test plates with the control plates, the images show a sharp decrease in bacterial colony density and a change in growth morphology. This can be scientifically explained by the integration of the nanomaterial with the nutrient medium, which directly interfered with the bacteria's biological processes from the very first moments of colonization. Visually, the discontinuous and irregular growth observed in the images indicates a state of severe cellular stress experienced by the bacteria, preventing them from forming a continuous biofilm as seen in the control sample. This superior inhibitory effect can be attributed to the action of the nanomaterial. The combination of the biological and nanocomponents is as follows:

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The role of Lactobacillus metabolites: Lactobacillus bacteria produce organic acids and bacteriocins that destabilize the cell membrane of resistant bacteria, making it more permeable. This high permeability allows silver nanoparticles (AgNPs) to easily enter the cell, where they bind to thiol (-SH) groups in proteins and enzymes [43][44]. disrupt DNA replication, ultimately leading to programmed cell death [45][46]. The specific bacterial response can also be observed that according to Gram-positive bacteria (*Staphylococcus*), These were the most affected due to the ease with which their porous peptidoglycan cell wall could be penetrated by the nanoparticles after weakening. With *Lactobacillus*. In case of Gram-negative bacteria (*Pseudomonas, Acinetobacter*): Despite possessing a complex outer membrane, the images demonstrated the compound's effectiveness in inhibiting them. This indicates that the biosynthesized silver nanoparticles possess a high affinity for binding to the surface of these cells and penetrating their defenses [47].

Table (7) Percentage inhibition of bacterial growth using a 20 mM concentration of the nanomaterial.

Visual observations	Growth inhibition percentage at a concentration of 20 mM	Resistant bacteria type
Near-total disappearance of colonies	95	*Staphylococcus aureus*
Very pale and discontinuous growth	90	*VRE (vancomycin-resistant enterococci)*
Very small and scattered colonies	85	*Acinetobacter baumannii*
A significant decrease in numerical density	80	*Pseudomonas aeruginosa*
marked inhibition compared to the control	78	*Klebsiella pneumonia*
Resistance	0	Control

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Figure (6) Percentage inhibition of bacterial growth using a 20 mM concentration of the nanomaterial.

Silver nanoparticles synthesized by *L. reuteri* bacteria exhibited broad-spectrum biological activity against multidrug-resistant bacterial isolates. These results are consistent with previous studies on biosynthesized nanoparticles. The high sensitivity of the Gram-positive bacteria is attributed to differences in cell wall structure. This observation, along with data on biofilm inhibition and the minimum inhibitory concentration, suggests the potential for developing a topical antimicrobial. Limitations: Small sample size, in vitro study design. *No cytotoxicity/biological effects data available.

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Conclusions: The green biosynthesis of silver nanoparticles using *Lactobacillus reuteri* bacteria demonstrated significant in vitro antibacterial activity against multidrug-resistant pathogens found in burns and ulcers. This antibacterial effect is attributed to membrane rupture, oxidative stress, and the interaction of the silver nanoparticles with bacterial proteins and nucleic acids. Further studies are needed to confirm the safety of this technique, its ability to disrupt biofilms, and its therapeutic potential in vivo.

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