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Effect of Biosynthesized Zinc Oxide Nanoparticles Against MDR Pathogenic Bacteria Isolated from Wounds and Burns

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Abstract: This study aimed to detected the antibacterial and antivirulence properties of zine oxide nanoparticles (ZnO-NPs) nanoparticles biosynthesized by *Lactobacillus salivarius* against bacterial infections from wounds and burns such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Acinetobacter baumanii* and *Staphylococcus aureus*. By calculating the minimum inhibitory concentration (MIC), the antibacterial activity was assessed. The results indicated that the MIC varied from 12.5 to 50 mg/ml. The anti-virulence factors were also found to be effective against the studied bacteria's hemolysin, urease, and biofilm formation. Results indicated that after being used in treatment with biosynthesized ZnO nanoparticles, the capacity to produce urease and hemolysin was reduced. The best effect was observed in biofilm formation after 72 hours for all isolates, with inhibition(14.07, 25.85, 41.08, 52.85, 46.43, 55.1, 36.9, 43.22)% for each of isolates *P. aeruginosa* (P8), *P. aeruginosa* (P11), *A. baumanii* (A3), *A. baumanii* (A9), *K.pneumoniae* (K5), *K.pneumoniae* (K6), *S .aureus* (S3), *S. aureus* (S9) respectively.

Keywords: ZnO nanoparticles, Biosynthesis , *Lactobacillus salivarius* , Virulence factors, Inhibition activity.

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Introduction

Nanotechnology is the newest and most proven technology in various industries, including food processing, chemical, mechanical, and pharmaceutical fields (1). Nanoparticles (NPs) are tiny materials that have (one to one hundred) nm size ranges, they were classified into different categories based on their sizes, forms, and other characteristics. Some of the categories include fullerenes, metal nanoparticles, ceramic nanoparticles, and polymeric nanoparticles. These properties have led to a multitude of commercial and domestic uses, including imaging, medicinal, catalysis, and environmental applications(2). The Zinc oxide nanoparticles (ZnO-NPs) are exciting inorganic materials (1), ZnO-NPs have many distinctive features in these nanomaterials, ZnO has been explored extensively and proven antibacterial activity against a wide range of bacteria. Moreover, ZnO is a cheap, readily available, and biocompatible substance. Additionally, this oxide offers certain useful advantages over commonly used

Ag NPs, including low production costs and photochemical stability (3). Zinc oxide nanoparticles (ZnO-NPs) are used in various industries such as cosmetics, medicine, concrete, antimicrobial textiles, cancer prevention, and antibacterial agents. This is due to their ability to produce reactive oxygen species (ROS) and trigger cell death (apoptosis)(4). Since microorganisms are easily cultured without being restricted by seasonal or geographic conditions, the biological synthesis of NPs using microorganisms has garnered more interest compared to plant-based NP production. This is because microorganisms seem to be a more environmentally sustainable method of producing NPs(5). The process of creating nanoparticles by biological synthesis involves the utilisation of enzymes, proteins, DNA, lipids, and carbohydrates by viruses, bacteria, fungus, algae, and plants, Metal-reducing bacteria are discovered to be environmentally benign catalysts for both materials production and bioremediation through the anaerobic respiration which includes a process that transfers electrons from reduced organic to oxidized inorganic substances, which facilitates the creation of crystals and nanoparticles and aids in bioremediation procedures. Additionally, biomolecules released by bacteria were employed as stabilizers for the creation of nanoparticles. Typically, the formation of nanoparticles occurs in the same manner as metal ions become lodged on the outside or inside of microbial cells, after that the presence of enzymes, the trapped metal ions are reduced to nanoparticle size (6,7). The current study aimed to study the antibacterial and antivirulence factors (Biofilm, urease and hemolysin) of biosynthesized ZnO-NPs by *Lactobacillus salivarius* on pathogenic bacteria isolated from wounds and burns.

Materials and methods

Microorganism

Forty-four pathogenic bacteria were isolated from wounds and burns using microscopic, cultural, and biochemical tests as per (18) and the Vitek2 system.

Detection of virulence factors:

Urease production

To determine the ability of pathogen bacterial isolates to produce urease enzyme it was used of urea agar indicated a positive result by a change of color from yellow to pink after 24 h at 37 °C (8).

Hemolysin production

Examined isolates were grown on blood agar using the streaking method. The purpose of this test is to identify hemolysin enzyme production. It was subsequently incubated for 24 hours at 37 °C, a hemolysis surrounding the colony indicated positive result (9).

Biofilm formation

Bacterial isolates were tested to determine if they were able to produce biofilms using the Microtiter plate method as described by (10,11). To perform the test, 180 µl of brain heart infusion broth with 2% sucrose was added to each well of 96 flat bottom well microtiter plates. Then, 20 µl of an overnight bacterial culture that was equal to 0.5 McFarland standard was added to each well as an inoculant. The covered microtiter plate was incubated for 24 hours at 37°C and sealed with parafilm. Next, the wells were washed three times with PBS (pH 7.2) to eliminate any detached bacterial cells. The wells were then allowed to dry for 15 minutes at room temperature before adding 200 µl of crystal violet (0.1%) for 20 minutes. The crystal violet

solution was removed, and the wells were rinsed three times with PBS (pH 7.2) to remove any unbound dye. After the wells were allowed to dry at room temperature, they were extracted twice using 200 µl of 95% ethanol. Finally, an ELISA reader was used to measure the absorbance at 630 nm for each well. The biofilm formation of each isolate was categorised into the following categories based on the absorbance values: (OD ≤ ODc (Non), ODc < OD ≤ 2 ODc (Weak), 2 ODc < OD ≤ 4 ODc (Moderate), 4 ODc < OD (High)(7).

Antibiotic susceptibility test

The described the Kirby-Bauer method, which was used to conduct an antibiotic susceptibility test for ten distinct antibiotics. To make the bacterial suspension, four to five colonies of each bacterial isolate were taken from the original culture and suspended in four millilitres of normal saline in a test tube. The turbidity was then adjusted to get about 1.5 × 108 CFU/ml (0.5 MacFarland standard). A sterile cotton swab was used to evenly spread a portion of the bacterial suspension over the surface of the Mueller-Hinton agar medium. After that, the agar was left to dry completely at room temperature. Using sterile forceps, antibiotic discs were firmly pressed onto the agar to ensure that they made contact with it. The plates were then turned over and left to incubate for a full day at 37°C. The isolate was determined to be either sensitive or resistant to a specific antibiotic based on the measurement of the inhibition zones that formed around the discs using a metric ruler.

Biosynthesis and characterization of ZnO Nanoparticles

Biosynthesis and characterization of ZnO-NPs were done in previous study (12), the ZnO-NPs biosynthesized by *Lactobacillus salivarius* and characterized using X-ray diffraction (XDR). The morphology and structure of ZnO-NPs were observed by using Fourier Transform Infrared, Spectroscopy (FTIR) and Scanning electron microscopic (SEM).

Antibacterial activity of synthesized ZnO nanoparticles

ZnO nanoparticles' antibacterial activity was evaluated by measuring their minimum inhibitory concentration (MIC) values. The MIC has the lowest concentration of ZnO nanoparticles and is expected to show no visible growth after a certain period of time. The MIC was determined using the microdilution method against bacterial strains of *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*, as described by (12). In summary, a stock solution of ZnO nanoparticles, which were biosynthesized by *L. salivarius*, was used. First, 125 µl of sterile broth (Muller Hinton) was added to the first column of a 96-well microtiter plate. The remaining wells were also filled with 125 µl of Muller Hinton sterile broth. Then, 125 µl of a ZnO nanoparticle solution (100 mg/ml) in PBS (phosphate-buffered saline) was added to the first column and mixed with the media. This gave a concentration of 50 mg/ml of ZnO nanoparticles. Subsequently, 125 µl was transferred to the next wells, discarding 125 µl of the mixture in the last column, resulting in a final volume of 125 µl in each well. Then, 2.5 microliters of an overnight growth (1.5 x 108 CFU/ml) of pathogenic bacterial isolates were added to each well. The microtiter plates were covered and kept at 37 °C for 24 hours. The concentration at which no visible growth occurred was considered as the

minimal inhibitory concentration (MIC).

Effect of biosynthesized ZnO nanoparticles on bacterial virulence factors production

Effect on urease production

ZnO-NPs biosynthesis was evaluated using the probiotic bacteria *L. salivarius*, which has been shown to prevent pathogenic bacteria involved in burn and wound infections from forming urease. In the experiment, urea agar was treated with 0.1 ml of ZNO-NPS (sub-MIC) and left to dry at room temperature. Following the inoculation of pathogenic bacteria onto the agar plate, it was incubated for 24 hours at 37 °C. According to (13), the appearance of pink colonies on the urea medium suggested that the pathogenic bacteria were producing urease enzymes, but the medium's yellow colour suggested that urease enzyme synthesis was absent.

Effect on production of hemolysin

ZnO-NPs biosynthesized was measured the effect by using the probiotic bacteria *L. salivarius* which has been found to inhibit hemolysin production by pathogenic bacterial isolates from wounds and burn infections. The blood agar is used in accordance with the following described in (14). The probiotic bacteria *L. salivarius* has been found to inhibit the formation of hemolysin production. The blood agar dish's surface was coated with 0.1 ml of ZnO-NPs (subMIC), which was then allowed to dry completely at room temperature. Following that, the blood agar plate was inoculated with the pathogenic bacteria inoculum and incubated for 24 hours at 37 °C.

Effect on biofilm formation

ZnO-NPs biosynthesized was measured effect by using the probiotic bacteria *L. salivarius*, which has been found to have an antibiofilm effect against isolates of bacterial wounds and burn infections (10). The microtitration plate method was used, where for each bacterial strain, 100 µl of bacterial suspensions were first placed in a well containing 2% sucrose brain heart infusion broth. Then, 100 µl of subMIC ZnO nanoparticles were added to the same well. The other wells were filled with 180 µl of brain heart infusion broth with 2% sucrose and 20 µl of bacterial growth suspension as a control. The plates were incubated at 37°C for 24 hours. After gently washing the microplates twice with deionized water to remove the non-adherent cells, they were dried at room temperature. Next, the microplates were stained for 20 minutes with a 200 µl crystal violet solution and then dried for 15 minutes at room temperature. For each well, 200 µl of (95%) ethanol was added after staining and any leftover stain was removed by washing with deionized water. The absorbance at 630 nm was then measured using an ELISA Reader device for each test-control well. A computation was used to determine that ZnO nanoparticles could prevent pathogenic bacteria from forming biofilms (15). The inhibition percentage of biofilm formation was calculated using the following formula: Inhibition percentage of biofilm formation = Control OD - Treatment OD/ Control OD ×100. Anti-biofilm estimates were used to determine the rate of bacterial biofilm formation that was inhibited compared to the control wells, which were reported at 100% to indicate that ZnO nanoparticles were absent. Conversely, data negative percentages showed that ZnO nanoparticles did not suppress the formation of biofilms.

Results and discussion

Collection and identification of isolates

It was diagnosed forty four isolates from pathogenic bacteria were isolated from wounds and burns. The isolates were detected through microscopic test, cultural test and biochemical test according to (18) and Vitek2 system and isolates included ((13) from *P. aeruginosa* , (11) from K. pneumonia , (10) from *A. baumannii* and (10) from *S. aureus*)

Virulence factors Detection

Urease enzyme production

Showed in Table (1), 6 from 13 isolates of *P. aeruginosa*, 7 from 11 isolates of *K. pneumoniae*, 6 from 10 isolates of *A. baumannii* and 7 from 10 isolates of *S. aureus* were urease positive through the hydrolysis of urea products by the Urease enzyme, which catalyses the hydrolysis of urea into ammonia, the final step of urea catabolism, causing the media's colour to change from yellow to pink. The urease enzyme was produced from several digestive and urinary tract pathogens, and it catalyzes the hydrolysis of urea to ammonia and carbamate and hydrolysis to ammonia and carbonic acid and causes to increased PH. The formation of gastric ulceration and urinary stones (16). There are many virulence factors in *K. pneumonia* which cause pathogenesis such as formed capsular, pili, polysaccharides and including them urease) (17). It found virulence factors of *P. aeruginousa* played roles in the pathogenicity, such as toxin A, alkaline protease (aprA), elastase, and many exoenzymes (18).

Production of hemolysin

All isolates of *S. aureus* produced hymolysin, 12 from 13 *P. aeruginosa*, 9 from 11 *K. pneumonia*, and 7 from 10 *A. baumannii* isolates produced hymolysin (Table 1). The toxin that attacked the membranes of erythrocytes and caused cell lysis was called hemolysin (19). Numerous types of toxins were discovered to be secreted by *S. aureus*; these include hemolysins (alpha, beta, gamma) and Panton-Valentine Leukocidin (PVL) to the efflux of vital molecules and metabolites and identified Hemolysins lead to iron scavenging, by have leucolytic properties, alpha-hemolysin damages a variety of host cells, such as erythrocytes, monocytes, endothelial cells, and epithelial cells, but beta - hemolysin affects immune cell function (20). Inhibition of the growth of *P. aeruginosa* was found by the uptake of gallium–protoporphyrin IX GaPPX throught Inhibition of the uptake of iron by bacteria (21).

Table (1): Urease and hemolysin production of wounds and burns bacterial isolates.

Bacterial isolates	Urease	Hemolysin
Pseudomonas aeruginosa	6 (13)	12(13)
Klebsiella pneumonia	7 (11)	9 (11)
Acinetobacter baumanii	6(10)	7 (10)
Staphylococcus aureus	7 (10)	10(10)

Biofilm formation

The microliter plate method was used to detect the biofilm formation ability of pathogenic bacteria isolated from wounds and burn infections, the potential of forty four isolates to biofilm formation was measured us by comparing O.D values of the stained attached cells. The result showed in the table (2) the ability of gram negative

produce strong biofilm (*P. aeruginosa* 11 from 13, K. pneumonia 10 from 11, *A. baumannii* 7 from 10) isolates and gram positive *S. aureus* 8 from 10 isolates , and was produce moderate biofilm 1 isolates from (13 , 11, 10) for each of (*P. aeruginosa, K. pneumoniae, S. aureus*) respectively and 3 from 10 isolates for *A. baumannii* and it was weak producing biofilm 1 from (13, 10) *P. aeruginosa* and *S. aureus* respectively isolates but no biofilm formation in K. pneumonia and *A. baumanii*. A biofilm is one of the virulence factors and its about a community of microorganisms, such as bacteria that can live and reproduce and is known as a colony (22). Biofilms can be formed by a wide types of microorganisms, including both gram-positive and gram-negative (23).

Table (2): Detection of biofilm formation of pathogenic bacteria isolated from wounds and burns infections.

Bacterial isolates	Biofilm formation		
	Number of biofilm bacterial isolates (number of total bacterial isolates)		
	Strong	Moderate	Weak
Pseudomonas aeruginosa	11 (13)	1 (13)	1 (13)
Klebsiella pneumonia	10 (11)	1 (11)	0(11)
Acinetobacter baumanii	7 (10)	3 (10)	0 (10)
Staphylococcus aureus	8(10)	1(10)	1(10)

Antibiotic susceptibility test

All isolates of *P. aeruginosa, K. pneumoniae, A. baumannii*, and *S. aureus* underwent an antibiotic susceptibility test using the disc diffusion test, which involved ten different antibiotic classes: carbapeneme class (imipenem); cephalosporin (ceftazidime and cefotaxime); penicillins (carbencillin); monobactams class of beta-lactam antibiotics (aztreonam); aminoglycosides (Amikacin); quinolones (Ciprofloxacin), fluoroquinolone (Norfloxacin); tetracycline and oxytetracycline. The results were interpreted according to the recommendation.

Table (3): Antibiotic resistance for pathogenic bacteria isolated from wounds and burns.

Bacterial isolates	Antibiotics									
	IMP	ATM	NOR	CTX	CAZ	T	AM	OT	PY	CIP
	Number of resistance isolates (number of total isolates)									
Pseudomonas aeruginosa	9 (13)	11(13)	7 (13)	10(13)	9(13)	12(13)	7(13)	10 (13)	13(13)	10(13)
Klebsiella pneumonia	6(11)	11(11)	8(11)	10(11)	7(11)	9(11)	6(11)	9 (11)	10 (11)	9(11)
Acinetobacter baumanii	8(10)	8(10)	7(10)	10(10)	8(10)	10(10)	4(10)	6(10)	8(10)	8(10)
Staphylococcus aureus	7(10)	8(10)	5(10)	6(10)	7(10)	7(10)	9(10)	7(10)	8(10)	5 (10)

IMP: impenem, ATM: Aztreonam, NOR: Nnorfloxacin, CTX: Ceftaxime, CAZ: Ceftazidime, T: Tetracycline, AM: Amikacine, OT: Oxytetracycline, PY: Carbencilline, CIP: Ciprofloxacin.

Based on how they prevent bacterial cells from forming cell walls, depolarizing, their membranes, synthesizing proteins, synthesizing nucleic acids, and avoiding metabolic processes in bacteria, antibiotics can be categorised into a wide range of classes(24). Gram-negative bacteria are more resistant to antibiotics than gram-positive bacteria, according to numerous studies and the reason about for horizontal gene transfer (HGT) and bacterial chromosomal DNA mutations(25). World Health Organization (WHO) found that three out of seven of the most resistant bacteria were *Klebsiella pneumoniae*, *E. coli*, and *Staphylococcus aureus*. And found the genetic changes and gain of mutation helped bacteria to survive in the new environments it is called horizontal gene transfer (HGT); this divides antibiotic resistance mechanisms into four groups (inactive the of drug, limiting drug. Altering drug target, and drug efflux were high levels(26). The reasons carbapenem-resistant in *P. aeruginosa* and *A. baumannii* were related to extended-spectrum β-lactamase (ESBL) and carbapenem-resistant *K. pneumoniae* and *Enterobacter spp* (27). A study claims that gram-negative bacteria are resistant to many antibiotics because of their outer membrane, including hydrophilic antibiotics like β-lactams that go through diffusion pathways and porins, and vancomycin, which can't go through the outer membrane because of its structure, which stops it from using any of these passageways (28).

Antibacterial Activity of Synthesized ZnO Nanoparticles

Bioynthesized zinc oxide's antibacterial activity was assessed against a range of clinical isolates from burns and wounds. The manufactured nanoparticles' minimum inhibitory concentration (MIC) varied from 12.5-20% mg/ml. The variety in the genus and species under test is probably the cause of the variation in the MIC of ZnO nanoparticles.

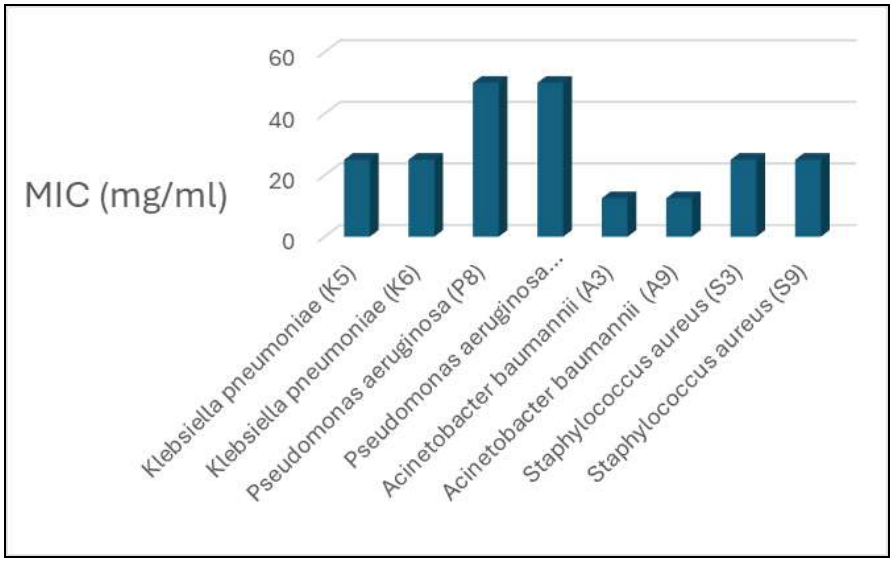

Figure (1): MIC (mg/ml) of zinc oxide nanoparticle (ZnO NPs) synthesized *Lactobacillus salivarius* against MDR wounds and burns bacterial isolates.

The role of lactic acid bacteria (LAB) in the synthesis of NPS has many factors (non –pathogenic and high production of various enzymes), and the release of Zn from ZnO NPs is an essential factor in antimicrobial through inhibiting a number of bacterial cell activates such as (bacteria metabolism, active transport and enzymes activity)(5). The NPs were antibacterial and active against several gram negative bacteria (*Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhi*) (24), ZnO NP has a high growth inhibition effect against *S. aureus* and *E. coli* bacteria (29). The use of transmission electron microscope (TEM) analysis in therapy bacteria by MIC of ZnO NPs, the result showed effects on the bacterial cell wall and caused damaged in cell membrane(30).

Results showed the effect of ZnO NPs against Gram negative more than Gram positive bacteria , it was showed the role of silver and zinc oxide nanoparticles with Pichia fermentans JA2 had antibacterial effect but the nanoparticles cause, the damage to microbes by the increased generation of reactive oxygen species (ROS) (31).

Gram-negative bacteria have a weaker peptidoglycan covering, which makes them more sensitive to nanoparticles. This is because interactions between the nanoparticles and the cell membrane are less effectively blocked by thinner peptidoglycan layers. The content, thickness, and structure of the bacterial cell wall affects the impact of nanoparticles when they come into direct contact with the bacteria. One crucial step in the entire process of causing bacterial cell death is the inhibition of enzymes by nanoparticles(32). When used against *A. baumannii*, which is resistant to carbapenem, ZnO-NP showed good antibacterial efficacy. The generation of reactive oxygen species is thought to be the mechanism of action of zinc oxide. This increases membrane lipid peroxidation, which leads to membrane leakage of reducing sugars, DNA, and proteins and decreases cell viability(33). And ZnO-NPs made from *A. niger* showed encouraging efficacy against *Klebsiella pneumoniae* carbapenemase KPC and improved wound healing, according to the study (34).

Effect of biosynthesized ZnO nanoparticles on bacterial virulence factors

Effect of biosynthesized ZnO nanoparticles on Biofilm formation

The result showed antibiofilm activity of ZnO nanoparticle biosynthesized by *L. salivarius* was reduced against *P. aeruginosa* (P8), *P. aeruginosa* (P11), *A. baumanii* (A3), *A. baumanii* (A9), *K.pneumoniae* (K5) *K.pneumoniae* (K6) *S .aureus* (S3), *S. aureus* (S9). After 72 hours, it was observed that ZnO-NPs showed the highest level of biofilm inhibition it was (14.07, 25.85, 41.08 , 52.85, 46.43, 55.1, 36.9, 43.22) respectively , but the biofilm inhibition in 48 hour it was moderate it was (11.81 , 13.91 , 34.11, 44.83 , 33.9 , 34.51, 27.52 , 23.97) respectively and biofilm inhibition in 24 hours it was (10.45 , 15.38 , 21.43 , 9.375 , 19.82 ,24 , 21.05) but no found inhibition biofilm formation in *P. aeruginosa* (P8).

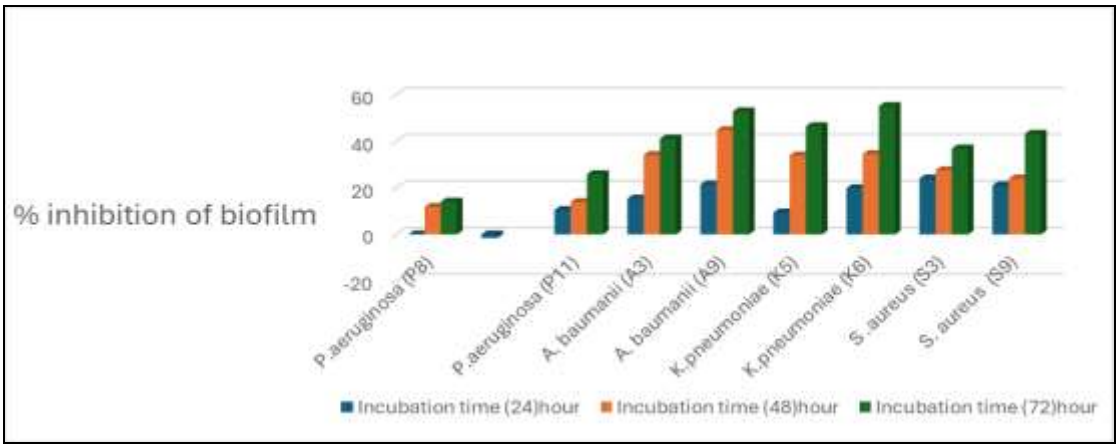

Figure (2): Antibiofilm effect of zinc oxide nanoparticle (ZnO NPs) synthesized *Lactobacillus salivarius* against MDR bacterial isolates from wounds and burns.

Antibiofilm activity of ZnO-NPs showed the role in the inhibition of *S. aureus* and four bacterial isolates from a number of clinical isolates endotracheal aspirate, burn, wound and ear infection, and the affected of ZnO-NPs in exopolysaccharide was played the role about adhesion to the host cell and the formation of biofilm and showed the accumulation of ZnO NPs in outer membrane trigger Zn release, and was caused effected in damaged of cell membrane and protein formation resulting death of bacterial cell (35). There are two reasons to make it that microbial nanoparticle synthesis has more benefits than other chemical and physical methods because of the two possible mechanisms for the antibacterial action, the first being the creation of increased levels of ROS, primarily hydroxyl radical and singlet oxygen, which destroy the bacterial cell wall, second nanoparticles on the surface of bacteria or their aggregation in the periplasm or cytoplasm cause disruption of cellular function and disarray in the membranes (36).

Valadbeigi *et al.* (37). found that using SMIC concentrations of ZnO NPs showed antibiotic activity against biofilm-producing *P. aeruginosa*. They were able to inhibit the formation of biofilms in bacteria at these concentrations (28). Discovered that ZnO NPs biosynthesized from Olea europaea (common olive) had a significant impact on the growth of *P. aeruginosa* and its biofilm formation (38). observed a 98% eradication of biofilms in *K. pneumoniae* after treatment with ZnO NPs for 24 hours.

Effect of biosynthesized ZnO nanoparticles on hemolysin

The study focused on the effect of ZnO nanoparticles synthesized by *L. salivarius* on the formation of hemolysis by pathogenic bacteria that cause infections in wounds and burns. The results revealed that the biosynthesized ZnO nanoparticles were affective against all bacterial hemolysins except for one strain of *P. aeruginosa* (P8) and two strains of *K. pneumoniae* (K5 and K6) isolates.

Table (4) Effect of zinc oxide nanoparticle (ZnO NPs) synthesized by *Lactobacillus salivarius* on hemolysin production of MDR bacterial isolates from wounds and burns

Bacterial isolates	Before treatments, Hemolysin	After treatments, Hemolysin
***Pseudomonas aeruginosa* (P8)**	+	+
***Pseudomonas aeruginosa* (P11)**	+	-
***Klebsiella pneumoniae* (K5)**	+	+
***Klebsiella pneumoniae* (K6)**	+	+
Acinetobacter baumannii (A3)	+	-
Acinetobacter baumannii (A9)	+	-
***Staphylococcus aureus* (S3)**	+	-
***Staphylococcus aureus* (S9)**	+	-

+: hemolysin positive - : hemolysin negative.

Hemolysin is an exotoxin that is highly effective in lysing a variety of host cells, including erythrocytes, macrophages, monocytes, endothelial cells, and epithelial cells. The lysis of RBCs induced by *S. aureus* was reduced when ZnO-NPs were added to the bacteria, and this resulted in a significant decrease in blood hemolysis when compared to untreated *S. aureus* control (35).

Effect of biosynthesized ZnO nanoparticles on urease

The study focused on the effect of ZnO nanoparticles synthesized by *L. salivarius* on the formation of urease by skin infection bacterial isolates. The results revealed that the biosynthesized ZnO nanoparticles was effected against all bacterial urease production except two of *K. pneumoniae* (K5, K6) isolates.

Table (5): Effect of zinc oxide nanoparticle (ZnO NPs) synthesized by *Lactobacillus salivarius* on urease production of MDR bacterial isolates from skin infection.

Bacterial isolates	Before treatments, urease	After treatments, urease
***Pseudomonas aeruginosa* (P8)**	+	-
***Pseudomonas aeruginosa* (P11)**	+	-
***Klebsiella pneumoniae* (K5)**	+	+
***Klebsiella pneumoniae* (K6)**	+	+
Acinetobacter baumannii (A3)	+	-
Acinetobacter baumannii (A9)	+	-
***Staphylococcus aureus* (S3)**	+	-
***Staphylococcus aureus* (S9)**	+	-

+ : Urease positive - : Urease negative.

Demonstrated how the water extract-activated ZnO-NPs inhibited the bacterial strains *S. aureus*, *E. coli*, and *P. multocida*, as well as the fungal strains *F. solani*, *A. parasitic*, and *A. niger*. Urease inhibition with *C. gigantea* flower extracts exhibits significantly higher activity when three compounds (methanol, leaf extract, and acetone extract of flower) are used. Additionally, urease inhibition with zinc nanoparticles is achieved through binding with the sulfuryl group present in the urease's active site, which reduces catalytic factors (39).

Conclusion

This study concluded the effectiveness of biosynthesized ZnO nanoparticles by *Lactobacillus salivarius* against growth and virulence

factors of wounds and burns bacterial isolates.

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