

Article

A comparative study of the effect of nanoparticles and antibiotics on the antimicrobial susceptibility of antibiotics for some bacteria isolated from wounds and burns infections.

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Abstract

This study aimed to investigate the isolation and identification of potential bacteria present in burns and wound infections. 100 pus types were taken from many patients who existed in the hospital of Babylon province from November 2021 to February 2022; the positive culture was 80% of the total isolates, while the negative culture was 20% of the total isolates. After the macroscopic, microscopic and biochemical tests, the bacteria were isolated from burn and wound swabs. Antibiotic susceptibility testing of the isolates was done using the disc diffusion method in conformity with McFarland standards. MDR of different types of antibiotics was exhibited from bacteria isolated in actual research. Statistical analysis using chi-square test The central objective of this research is to use different concentrations of Zinc oxide nanoparticles to obtain the optimum antibacterial concentration. Males (37.5%) and females (62.5%), with ages ranging from 5 to 55 years. The high percentage was less than ten years old, while the lowest was between (and 50_60). Where Gram-negative bacteria were most common, the concentration of Zinc oxide nanoparticles was 100 µg/ml. 150 µg/ml affects gram-positive and gram-negative growth by measuring the diameter of the inhibition zone on the growth culture that is artificial to Gram-positive and Gram-negative bacteria throughout the measuring inhibition zone around the wells. We observed that the diameter of the inhibition zone increased in concentration (150) µg/ml more than the concentration (100) µg/ml. The study showed women of positive growth and the ages compared to males. Multi-Drug Resistance bacteria. Zinc oxide nanoparticles have critical applications as they are antibacterial and effectively dress for burns and wounds .

Keywords: Gram-positive Bacteria, Gram-negative bacteria, Wounds, Burns, Antimicrobial sensitivity, Multi-Drug Resistant, nanoparticles.

Introduction:

The revelation of dermatological material resulting in skin safety and wound care damage delivers a humid, heartfelt, and nutritional situation favorable to forming colonies of different microorganisms and producing new colonies. The wound has developed into an infected condition and will likely be affected by numerous microbes and condiments.¹

Exposing the skin to one or the other trauma, surgery, or burns leads to inflammation of the skin and peripheral soft tissue, which produces secretions consisting of dead white blood cells, cellular rulers and dead tissues.²

Wound and burn contamination results from harmful microorganisms in blood or other tissues, limb damage, elongated hospital vacations, and more significant expenditures. It is accountable for meaningful humanoid death and universal diseases,³ while it was considered one of the most significant common hospital injuries.⁴

The wound may be diseased through various microorganisms, encompassing bacteria, fungi parasites, and viruses.⁵

Wound and burn contaminations were described through brutally limited infection following tissue damage. This led to comprehensive microbial diseases by the several microorganisms that can produce toxicity connected to infestation of infectious microbes. Several of the communal etiological causes accountable used for producing microbial infections are bacteria like (*Staphylococcus aureus*), (*Streptococcus pyogenes*), (*E. coli*), (*Klebsiella* spp.), (*Proteus* spp.), and (*Pseudomonas* spp).⁶

Antibiotics are of great value in the management and prevention of infections, thus preventing infection, the time of antibiotic administration, and the optimal selection of antimicrobials. Therefore, the periods of antibiotic administration and the appropriate dose have been determined, which is essential in reducing wound infection.⁷

Nano-zinc oxide or (ZnO) nanoparticles have established numerous implementations in everyday life, such as drug transfer, foundations, and health strategies, due to their authoritarian antimicrobial influences on a broad spectrum of different microorganisms.⁸

ZnO-NPs are considered the most broadly usage Nano compounds that are U.V. absorbers in fabrics,⁹ wastewater management uses,¹⁰ and resurrection controllers.¹¹ Zinc oxide nanoparticles have perfect therapeutic effects to become successfully used in place of identification of nanoparticles properties, infectious cause, bio-imaging, drug transfer, and in carcinoma management, etc.¹²⁻¹³ Related to typical cells, ZnO-NPs show a suitable capability to extinguish tumor cells in humans¹⁴. They may then become a possible contestant for anti-cancer events.¹⁵⁻¹⁶ The possible cytotoxicity purposes of (ZnO-NPs) had been connected with apoptosis existence¹⁷. Furthermore, original methods are wanted for medicinal requests of ZnO-NPs to be active and complete in bacteriological and antiseptic actions.¹⁶⁻¹⁸

Materials and Methods

Samples Collection:

One hundred samples were obtained from different patients who suffered from wounds- and burn infections 5 and 50 and were present in Babylon province from November 2021 to February 2022. Initial information of infected patients was recorded (age range from 5 to 50 and sex male and female). Usual bio-chemical trials were consumed to identify the causative agent of pathogens. Determination of positive bacterial culture that was causing causative agent of the pathogen may be classified through dependent on the morphology possessions as colony size, form, coloring, nature of pigments, clearness, verge, rise and stability). Then, positive colonies might stain via gram stain to notify a confident color, nature, type of reactions, accumulation and certain intra-cellular compounds in accord with WC Winn.¹⁹

Biochemical Identification:

This current study included conducting biochemical tests to identify and diagnose the isolated bacterial species, which includes tests such as the Catalase test, Oxidase test, Coagulase test and IMVC test (Indole test, Methyl red test, Vogues- Proskauer test and Citrate utilization test).

Antibiotic susceptibility or Antibiotic Sensitivity test:

This test was completed by making sterile Mueller–Hinton agar and standardized as instructed by the manufacturer. The medium was dispensed into (petri plates) and allowable into the solid medium. The inocula were also prepared in test tubes concerning 0.5 McFarland's standard. The test isolates were inoculated on the Mueller-Hinton agar using a sterile swab stick. After that, an antibiotic disc was placed onto the inoculated plates and incubated at 37°C for 24 hours. The plates were read, and the inhibition zones were measured to the nearest mm.

The inoculums used in this experimentation were made ready by adding 3 into 5 samples of isolating colonies mature on a nutrient agar plate to 5 mL. Of antiseptic normal saline and connected within (1.5×10^8 cell/mL) MacFarland typical tube. With sterilized swabs, the sensitivity Muller Hinton medium was infected via rotating the swab overhead the surface of Muller Hinton agar. Previously utilizing sterilized forceps, the antimicrobial discs were placed on the inoculum and plates were incubated for 24 hours at 37°C using the disk diffusion technique, revealed via the Clinical and Laboratory Standards Institute CLSI.²⁰ Formerly, the zones of inhibition can determine the sensitivity strategy. Antibiotic sensitivity defined in connection with the inhibition areas was measured to evaluate the sensitivity strategy.²¹

Antimicrobial category	Antimicrobial agent	symbol	disk µg/ml	Sensitive	Resistant
Aminoglycosides	Gentamicin	GEN	10 µg	≥15	≤12
Quinolones and fluoroquinolones	Levofloxacin	LEV	5 µg	≥17	≤13
Quinolones and fluoroquinolones	Trimethoprim	TMP	5 µg	≥16	≤10
Beta-lactam	Amoxycillin	AMX	20 µg	≥20	≤19
Folate pathway inhibitors	Ciprofloxacin	CIP	5 µg	≥21	≤15
Penicillins	Piperacillin	PRL	10 µg	≥21	≤14
Quinolones and fluoroquinolones	Norfloxacin	NOR	10 µg	≥17	≤12
Carbapenems	Imipenem	IPE	10 µg	≥19	≤15
Penicillins	Ampicillin	AM	10 µg	≥17	≤13

Table 1. Antibiotics disks.

Preparation of ZnO Nanoparticles:

The zinc oxide (ZnO) nanoparticles were synthesized through a wet chemical method utilizing chemical materials such as zinc sulfate and sodium hydroxide precursors and soluble starch as a stabilizing agent. Preparation of soluble starch with a percentage (0.1%) in Different concentrations was liquefied in D.W. (500 ml) via a microwave oven. Zinc sulfate, 14.874 g (0.1 mol), was additional in the overhead mixture. Formerly, the solution was preserved beneath continuous stirring, consuming a magnetic stirrer to finally melt the zinc nitrate for some time (one hour). Later, the whole solution of zinc nitrate, 0.2 mol of sodium hydroxide solution, was added beneath continuous stirring, droplets via drop moving the vessel's walls. The situation was allowable to continue for 2 h next whole adding of sodium hydroxide. After the completion of the reaction, the final solution was

permissible to settle for overnight, and the supernatant solution was discarded carefully. The remaining solution was centrifuged at 10,000 rpm for 10 min, and the supernatant was rejected. Therefore, the achieved nanomaterials were washed away three times utilizing D.W. Washing was passed out to remove the byproducts and the too much starch bound within the nanomaterial. After washing, the ZnO nanoparticles were dried at 80°C for 24 hours. Throughout drying, comprehensive Zn (OH)₂ alteration into ZnO occurrence.²²

Fresh bacterial cultures to be tested for sensitivity to nanomaterial were prepared by growing them on a nutrient broth medium and incubating them at a temperature of 37°C for 24 hours. Then, each bacterial isolate was cultivated on a suitable medium by swab. Then, we drilled on the cultured medium with a sterile cork perforator and put 100µL in each hole for each of the concentrations used by the diffusion method in the culture medium and after the incubation period at 37°C for 24 hrs. We observed the results by measuring the area of the inhibition zone around bacterial growth. The antibacterial activity was evaluated against Gram-negative *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *E. coli* and *Proteus spp* and gram-positive *S. aureus* and *S. pyogenes*. The strains were cultured in L.B. broth overnight at 37°C in an incubator. Then, the bacterial species isolated was streaked on Muller Hinton agar and used different concentrations of zinc oxide nanoparticles, including (100 µg/ml and 150 µg/ml) and kept incubated at 37°C for 24 hrs. After the specified period, the regain of the inhibition zone was calculated by a ruler to evaluate the effect of nanomaterials on each bacterial species and within each concentration and plotted to quantify the antibacterial activity.

Statistical Analysis:

Study data may be investigated using SPSS variety 16 and Microsoft Office Excel 2007. Standard statistics may be identified, such as numerals and percentages. Fischer's exact assessment was used to relate the rate of incidence. A p rate of fewer than 0.05 was restrained significantly.

Results

Microbial identification:

One hundred pus samples were obtained from different patients who existed in some hospitals of Babylon province from November 2021 to February 2022; the positive culture was 80% of total isolates, while the negative culture was 20%. The results of the present study are revealed in Table 2.

The current study was dissimilar within research via²³ who exhibited positive culture with 43.7% of total isolates,²³ while another study showed 43.7% positive growth whereas 56.3% were growth negative of total isolates.²⁴

Sample Number	Growth	Number of samples	percentage% of samples
1	Growth- positive	80	80%
2	Growth- negative	20	20%
Total		100	100%

Table 2. Growth pattern of Bacteria.

In the current study, positive cultures formed 80 % of total isolates, including males and females, ranging from (5_55) years. The number of males was (30)samples of positive isolates with a percentage (of 37.5%), but the number of females was (50)samples of positive isolates with a percentage (62.5%). The high percentage was in age less than 10 years old, with (25%) of total positive culture,

while the lowest percentage was between (and 50_60) years old, with (11.25%) of total positive isolates. The results are shown in Table 3.

The actual study was comparable to another study, which showed that females had a high percentage of infection compared with males within a percentage (20.16%) of positive growth. This is dissimilar to another study in which the maximum number of isolates in the fifth decade, followed by the fourth decade-- a total of 100 cases of wound infection.²⁵ Possibly, the absence of edification, special- hygiene items and absence of sound- sustenance lead to microbial contamination in burns and wounds.

Age groups	Male	Female	No. of samples.	% of samples.
>10	7	13	20	25%
10_20	5	14	19	23.75%
20_30	3	7	10	12.5%
30_40	8	4	12	15%
40_50	5	5	10	12.5%
50_60	2	7	9	11.25%
Total	30	50	80	100%

Table 3. Age and gender distribution of growth-positive cases.

Out of a total bacterial isolate, (59) isolates (73.75%) were Gram-negative, and (21) isolates (26.25%) were Gram-positive bacteria. *Pseudomonas aeruginosa* was (25) isolates (31.25%), which consider the most common isolates of bacteria followed by *Klebsiella pneumoniae* was (18) isolates (22.5%), *Staphylococcus aureus* was (12) isolates (15%), *Escherichia coli* was (10) (12.5%), *Streptococcus pyogenes* was (9) isolates (11.25%) finally *Proteus spp* was (6) isolates (7.5%) as shown in table 4.

Organism	No. of isolate	% of total isolate
<i>P. aeruginosa</i>	25	31.25%
<i>K. pneumoniae</i>	18	22.5%
<i>S. aureus</i>	12	15%
<i>E. coli</i>	10	12.5%
<i>S. pyogenes</i>	9	11.25%
<i>Proteus sp</i>	6	7.5%
Total	80	100%

Table 4. Distribution of bacteria isolated.

The present study was dissimilar with numerous researchers of Africa, and diverse parts of the current study was dissimilar to another study by Obritsch et al. (2004), who reported that the rate of isolation of gram-positive bacteria 30 (54.54%) was more than that of gram-negative bacteria 25 (45.45%). The rate of gram-negative bacteria isolation from burn wounds was more than twice that of gram-positive.²⁶ the sphere with another study where exhibited *Staphylococcus aureus* was predominant that 26 isolates (47.27%) followed by *Escherichia coli* 10 isolates (18.18%), *Pseudomonas aeruginosa* 8 isolates (14.54%), *Streptococcus pyogenes* 4 isolates (7.27%), *Proteus sp*, 3 (5.54%) and *Klebsiella pneumoniae* 3 isolates (5.54%).²⁷

As well as where the most common organisms, *Pseudomonas aeruginosa* and *E. coli*, were measured as the principal bacteria isolates from wound and burn infections.²⁸

Also, Baba *et al.* (2016) showed in their study that *Staphylococcus aureus* 26 (47.27%), *Escherichia coli*, 10(18.18%), *Pseudomonas aeruginosa* 8 (14.54%), *Streptococcus pyogenes* 4(7.27%) and *Klebsiella pneumoniae* 3 (5.45%).²⁹

The current study was similar to another study that showed the positive results were 89 (89%) samples of total bacterial isolates and only 11 negative isolates of wound swabs in bacterial culture. *P. aeruginosa* was the most common pathogen isolates (31.46%), followed by *Klebsiella spp.* (22.47%) , *S. aureus* (20.22%) , *E. coli* (15.73%), and *Proteus spp.* (10.11%).³⁰, while the other study was dissimilar to the present study that showed Gram-negative bacteria were 158 isolates with a percentage (71.82%) more than Gram-positive bacteria were 62 with a percentage (28.18%). *Pseudomonas spp.* (34.55%) was best communal, followed by *Staphylococcus aureus* (21.36%), *Escherichia coli* (11.82%), *Klebsiella pneumoniae* (4.55%), *Streptococcus spp.* (0.91%).²³

Antibiotic susceptibility characterization:

An antimicrobial susceptibility test was tested, and this test showed that *Pseudomonas aeruginosa* was ultimately resistant to PRL (100%). In comparison (90%) of isolates exhibited resistance to IPE, (80%) of isolates revealed resistance to GEN and LEV (50%) of isolates formed resistance to AMX, (30%) of isolates exhibited resistance to TMP, finally (10%) of isolates resistant to CIP and NOR.

Klebsiella pneumoniae showed resistance to different types of antibiotics (90%) of isolates formed resistance to PRL, (70%) of isolates revealed resistance to GEN, (60%) of isolated resistant to LEV and NOR, (50%) of isolates appeared resistant to TMP, (40%) of isolates formed resistance into AM, (30%) of isolates showed resistance into AMX and (10%) of isolates resistant into CIP and IPE.

Escherichia coli was resistant to antibiotics as (90%) of isolates showed resistance to PRL, (80%) of isolates formed resistance to GEN, (75%) of isolates appeared resistant to AM, (70%) of isolates were resistant to IPE, (60%) of isolates formed resistance to LEV and NOR, (50%) of isolates appeared resistance to CIP, (40%) of isolates exhibited resistance to TMP and (10%) of isolates showed resistance to AMX.

Finally, *Proteus sp* was resistant to antibiotics, such as (70%) of isolates appeared resistant to NOR, (65%) of isolates were resistant to IPE, (60%) of isolates formed resistance to GEN, (55%) of isolates revealed resistance to LEV, (40%) of isolates exhibited resistance to PRL, (30%) of isolates showed resistance to CIP, (20%) of isolates resistant to TMP at last,(10%) of isolates showed resistance to AM, this percentages of the resistance to different types of antibiotics showed in table(5).

Antibiotics	<i>Pseudomonas Aeruginosa</i>		<i>Klebsiella Pneumoniae</i>		<i>Escherichia coli</i>		<i>Proteus sp</i>	
	S	R	S	R	S	R	S	R
GEN	20	80	30	70	20	80	40	60
LEV	20	80	40	60	40	60	45	55
TMP	70	30	50	50	60	40	80	20
AMX	50	50	70	30	90	10	100	0
CIP	90	10	90	10	50	50	70	30
PRL	0	100	10	90	10	90	60	40
NOR	70	10	40	60	40	60	30	70

IPE	10	90	90	10	30	70	35	65
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Table 5. Percentage sensitivity of some Gram-negative isolates to some antibiotics.

Furthermore, Gram-positive bacteria showed resistance to different types of antibiotics, such as *S. aureus*, in which (65%) of samples showed resistance to PRL, (60%) of samples were resistant to TMP and IPE, (50%) of samples appeared resistant to CIP (40%) of samples formed resistance into LEV, (30%) of samples revealed resistance into GEN and NOR. *S. pyogenes* which (65%) of samples showed resistance to TMP, (55%) of samples appeared resistant to IPE, (50%) of samples were resistant to PRL, (40%) of samples showed resistance to AMX, (30%) of samples exhibited resistance to LEV, (20%) of samples formed resistance into GEN and AM at last (10%) of samples resistant into CIP, the results as shown in table 6.

Antibiotics	<i>Staphylococcus aureus</i>		<i>Streptococcus pyogenes</i>	
	S	R	S	R
GEN	70	30	80	20
LEV	60	40	70	30
TMP	40	60	35	65
AMX	100	0	60	40
CIP	50	50	90	10
PRL	35	65	50	50
NOR	70	30	80	20
IPE	40	60	45	55

Table 6. Percentage sensitivity of some Gram-positive isolates to some antibiotics.

The current research was dissimilar with other research by ²⁴ who showed the antimicrobial susceptibility design of *Pseudomonas spp.* to diverse types of antibiotics such as Ciprofloxacin 68.4%, Gentamicin 35.5% and Imipenem 5% and *S. aureus* resistant to Ciprofloxacin 53.2, Gentamicin 36.2, Amoxycillin 89.4 and Vancomycin 0. ²⁴

Baha *et al.* (2016), who exhibited The percentage susceptibility results of *P. aeruginosa* and *Proteus sp.* isolates, were susceptible to some antibiotics but resisted Ciprofloxacin and Gentamycin. *K. pneumonia* resisted Ciprofloxacin (75%), and *S. aureus* isolates were highly susceptible to LEV (88%). In this situation of *S. pyogenes*, the samples revealed 100% susceptibility to CIP, C and LEV and hardly exhibited resistance to AMX (75%). ²⁹

Nanoparticles' effects on bacterial growth:

The present study exhibited ZnO nanoparticle's effects on microbial growth of different types of bacteria isolated from wounds and burns samples, which, including different concentrations of zinc oxide data, indicated that it could efficiently eliminate bacteria in vivo, which is payable to wound healing. The antibacterial activity was estimated against Gram-positive bacteria such as *S. aureus* and *S. pyogenes* and Gram-negative bacteria like *P. aeruginosa*, *K. pneumonia*, *E. coli* and *Proteus spp.*, whereas antibiotic rehabilitation is inefficient. In the current study, we prepared different concentrations of ZiO nanoparticles in the chemical method,

including 100 µg/ml and 150 µg/ml that were artificial to Gram-positive bacteria such as *S. aureus* and *S. pyogenes* throughout the measuring inhibition zone. We observed that the diameter of the inhibition zone increased in concentration 150 µg/ml more than the concentration of 100 µg/ml in the growing of bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*. In contrast, in concentrations of 100 µg/ml and 150 µg/ml, the inhibition zone of bacteria *Staphylococcus aureus* was 27mm and 30mm, respectively. While the inhibition zone of *Streptococcus pyogenes* in concentrations 100 µg/ml and 150 µg/ml were 20 mm and 25mm, respectively. As shown in table (7).

Concentration	<i>S. aureus</i>	<i>S. pyogenes</i>
100 µg/ml	27mm	20mm
150 µg/ml	30m	25mm

Table 7. Zone of inhibition values (mm) of different concentrations of ZnO-NPs of some Gram-positive isolates.

In the current study, we observed that the diameter of the inhibition zone increased in concentration to 150 µg/ml rather than the concentration of 100 µg/ml in the growth of Gram-harmful bacteria comprising *P. aeruginosa*, *K. pneumoniae*, *E. coli* and *Proteus spp*. In contrast, the inhibition zone in the 100 µg/ml concentration was 15mm, 20mm, 15mm and 10mm, respectively. While the inhibition zone in the concentration 150 µg/ml were 22mm, 25mm, 13mm and 11mm, respectively. As shown in table (8).

Concentration	<i>P. aeruginosa</i>	<i>K. Pneumoniae</i>	<i>E. coli</i>	<i>Proteus sp</i>
100 µg/ml	15mm	20mm	15mm	10mm
150 µg/ml	22mm	25mm	13mm	11mm

Table 8. Zone-inhibition values (mm) of different concentrations of (ZnO-NPs) of some Gram-negative isolates.

Discussion

Today, researchers present unique alternate antibacterial-like metallic nanoparticles, where metal nanoparticles, especially ZnO nanoparticles, are highly appreciated. Thus, many recent studies have shown that ZnO nanoparticles involve a probable antibacterial activity.³¹

The recent study is different from a different study by Shabnam *et al.*, which confirmed the bacteriostatic effect against *S. aureus* after treated ZnO nanoparticles, and it exhibited an inhibition zone of bacterial growth at a concentration of 125 µg/ml.³²

Similarly, Jones *et al.* established that zinc oxide nanoparticles process several usages, such as a bacteriostatic effect against different types of bacteria,³³. At the same time, the study of Zhang *et al.*³⁴ demonstrated that ZnO nanoparticles have bacteriostatic activity against the bacterial growth of *E. coli*.

The consequences of the current research differ from the results of the study of³¹, which exhibited that ZnO nanoparticles have inversely antibacterial activity associated with their size. Therefore, in the current study, ZnO nanoparticles with 10-30 nm sizes showed an increase in the antimicrobial activity of ZnO nanoparticles to treat wound burn infection. Numerous studies that are increasing angiogenesis³⁵ and wound remedial exhibited the essential application of ZnO nanoparticles.³⁶

It is worth noting and well known that high concentration zinc ions can negatively affect various bacterial activities in place of glycolysis, dermal lysis, transport of protons across the cellular membranes and acid broadmindedness that can elongate the lag phase of bacterial growth.³⁷

The results of the current study were dissimilar to another study. Their Inhibition zone of bacterial growth of Gram-negative and Gram-positive bacteria in concentrations 100 µg/ml and 500 µg/ml comprising *E. coli* were 11±0.22 and 13±0.26, respectively, and *Klebsiella Pneumoniae* were 10±0.2 and 11±0.22 respectively, *Staphylococcus aureus* were 10±0.2 and 12±0.24 respectively, and *Streptococcus pyogenes* were 8±0.16 and 9±0.18 respectively.³⁸

The present study was similar to Jalal *et al.*'s research, which achieved a durable antibacterial effect against *Escherichia coli* by increasing concentration. For example, as a result, an increase in H₂O₂ amount was created from the ZnO surface, thus leading to a fatal dose of bacteria.³⁹

Recent developments in nanotechnology, especially the capability to organize precision Nano particulates of any size and shape, have directed the development of resistant particles. Numerous studies showed that Nano particulates are used for bactericidal growth.⁴⁰

Conclusion:

The study showed that the bacterial infection of wounds and burns was more prevalent in women than males of positive growth. At less than ten years old, the isolated positive samples were more infected. The lowest percentage of bacterial infection was between 50 and 60 years, and the positive samples were Gram-negative.

In the present research, the significant numeral of Multi-Drug Resistance bacteria was instituted through the connective agent of wounds and burn infections. So, routine micro-biological analysis of the wound specimen and their antibiotic sensitivity testing were commended. It will be associated with microbial practitioners for empirical management of wound infection to decrease the range of bacteria resistance.

Zinc oxide nanoparticles have great potential in biomedicine and have critical applications as they are antibacterial and effective dressing for burns and wounds .

We observed that the diameter of the inhibition zone increased in concentration 150 µg/ml more than the concentration (100) µg/ml in the growing of bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, whereas in concentration (100) µg/ml and (150) µg/ml. Then, we observed that the diameter of the inhibition zone increased in concentration (150) µg/ml rather than concentration 100 µg/ml in the growth of Gram-negative bacteria.

Ethical Clearance

Scientific research is subject to the Research Ethical Committee in the service of research through moral approval for any environmental research and organized and extensive study, as well as research subject to the Ministry of Higher Education and Scientific Research in Iraq.

Acknowledgment

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Conflict of Interest

The authors declare there are no competing interests.

Author's Contribution

. Yusra A. Radeef conceived and designed the experiments performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared

figures and tables, authored or reviewed drafts of the paper, and approved the final draft.

. **Anmar Mahdi Kadhum AL-Maamori** performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and tables, and authored or reviewed paper drafts.

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Data Availability

The following information was supplied regarding data availability:

The raw data are provided in the Supplemental Files.

Ethics Statement

Scientific research is subject to the Research Ethical Committee in the service of research through moral approval for any environmental research and organized and significant study, as well as research subject to the Ministry of Higher Education and Scientific Research in Iraq.

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