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Evaluation of The Effect of Zinc Oxides Nanoparticles on Bacteria Activity And Biofilm Formation of Clinical *Acinetobacter Baumannii*

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Abstract :

This study was conducted in the Mustansiriyah University, College of Science, laboratories of Department of Biology , from (November 2024 to January 2025) . A total of (120) samples were collected from different clinical sources, including burns, wounds, and urine and blood In addition to environmental samples from the hospital environment . Al-Imamain Al-Kadhimiya Hospital, Al-Karkh General Hospital, and the Medical City's teaching laboratories were among the participating hospitals. Every sample was cultivated on MacConkey Agar and Blood Agar media and incubated for the whole night at 37°C. Only 27 isolates were identified as *Acinetobacter baumannii*. All these isolates were identified and purified by culture on chromium agar, biochemical tests, and the use of the 16S rRNA gene.

Zinc oxide nanoparticles were then biosynthesized from *Pseudomonas aeruginosa* and hydrated zinc sulfate (ZnSO4.7H2O). If zinc sulfate is reduced to form zinc oxide nanoparticles.

The properties of the resulting nanomaterial were studied using different devices, including UV, EDX , XRD, and SEM. The results of these devices showed up the formation of pure ZnO Nps in various shapes and sizes ranging between 20 to 100 nm . The ability of synthesized nanoparticles to inhibit *Acinetobacter baumannii* bacteria was studied at concentrations of (0.4, 0.6, 0.8, 1, 1.2, 1.4, and 1.6) mg/ml. It was found that the effectiveness of ZNO NPS increased with increasing concentrations The highest percentage was at a concentration of 1.6 (94%).

The efficiency of the synthesized nanomaterial in inhibiting some virulence factors was tested. The ability of ZnO Nps On the Inhibition of biofilms formation in *Acinetobacter baumannii* bacteria was studied. A range of different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5) mg/ml were used. The concentration of 0.4 and0.5 showed the highest biofilm inhibition .

Keywords: *Acinetobacter baumannii*, Biofilm, ZnO nanoparticles

1. Introduction

A. baumannii is a rod-shaped, spherical, and tiny organism (coccobacillus). It's a name. The bacteriologist Paul Baumann [1]. It is becoming a more significant nosocomial infection and can infect people with .weakened immune systems as an opportunistic pathogen

A baumannii has been a harmless microbe since ancient times, but over the past three decades it has become an important and dangerous opportunistic pathogen that is widely spread in clinical settings, especially in hospital settings in global , because to its ability to develop and acquire multi-resistance to many commonly used antibiotics Therefore, the treatment of these bacteria has become more difficult in recent times [2]. The World Health Organization in 2017 published a list of pathogens that require new generations of antibiotics among these broad pathogens are (ESKAPE) *Enterococcus faecium*,. *Enterobacter* , *Pseudomonas aeruginosa*, *A. baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus)* which has been classified as (Priority status)[3]. These bacteria's genetic makeup enables them to possess a variety of antibiotic resistance strategies, such as the synthesis of enzymes, the formation of biofilms, the regulation of gene expression, and mutations [4].

A. baumannii is typically isolated from hospital surroundings, , Although other species of the genus are frequently identified in soil samples this contributes to the widespread belief that *A. baumannii* is also a soil bacterium [5].

A. baumannii is a pathogenic bacteria that causes problems on a global scale, causing a lot of infections, especially among people with immune compromised system and patients in intensive care units, including bacteremia, pneumonia infection , meningitis, wound and urinary tract infections, also skin infection ,leading to increase mortality rates of more than 40%. [6]

The pathogenicity of *A. baumannii* bacteria is because to present many virulence factors, including Biofilm, outer membrane proteins (Protein A Omp4), lipopolysaccharides, phospholipids, efflux pumps .

One of the most significant issues linked to elevated antibiotic resistance in *A. baumannii* is the formation of biofilm [7].

Because of their unique structure and the extracellular polymeric materials they contain, biofilms limit the penetration of antibacterial drugs. When treating illnesses brought on by bacteria that produce biofilms, physicians encounter numerous challenges. [8]. In addition to host epithelial cells, *A. baumannii* can form biofilms on a variety of surfaces, including abiotic surfaces like polypropylene and stainless steel [9].

Key biofilm related genes include bap (biofilm associated protein), csuE (part of the chaperone usher pathway), and others. All play important role in cell adhesion, biofilm formation, and structural stability [10] [11] .

Together, these elements form powerful biofilms that are extremely resistant to antibacterial treatments and immune cells.[12] [11] .

Biofilm-forming bacteria have been considered the most important lifethreatening pathogens in the world; the current study aims to find a safe material that can stop or even eliminate the formation of bacterial biofilm to reduce antibiotic resistance . The purpose of this study Looking for new materials (especially ZNO nanoparticles) that can reduce bacteria ability of gene expression of biofilm formation .

Nanoparticles are currently considered an effective alternative to antibiotics due to their high ability to eliminate multidrug resistant bacteria.

[13]A new range of possibilities has been made possible by the application of nanotechnology to create new nanoparticle for use in medical. [14]. A metal nanoparticle is regarded as an efficient antibacterial agent because of its numerous qualities that make it important for medical applications. As a result of its high biological activity, zinc oxide NPs have been the subject of much research. The most often utilized zinc nanoparticle is zinc oxide [15]. This is because it is more effective against pathogenic bacteria and has less toxic properties , they also have selective toxicity against bacteria [16] Nanoparticles possess many important properties, such as high catalytic activity, chemical and physical stability, and antibacterial activity and Some nanoparticles have antibiofilm activity [17]. Numerous studies have found effective antibacterial and antibiofilm effects of nanomaterials against various bacterial groups, such as *Salmonella enteritidis* , *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Listeria monocytogens* [18]. Given the growing importance of green technologies, nanoparticle have been synthesized from various sources, such as plants, algae, fungi, bacteria, and others [19].

2. Methodology

2.1 Clinical samples collection

This study was conducted in the Mustansiriyah University, College of Science, laboratories of Department of Biology . In the period from (November 2024 to January 2025)A total of 120 specimens were collected from different clinical sources, such as burns, wounds, blood, and urine, in addition to environmental samples taken from the hospital setting. Al-Imamain Al-Kadhimain Hospital, Al-Karkh General Hospital, and Teaching Laboratories in Medical City were the hospitals that were engaged. All samples were cultured on blood agar and MacConkey Agar media .

2.2 Identification of bacterial isolates

All isolates were diagnosed, depending on the Properties of the culture , microscopic characteristics and biochemical tests and molecular identification of isolates by 16s rRNA.

2.3. Nanotechnology :

2.3.1 ZnO NPs synthesis:

Nanoparticles were synthesized using the following method:

Pseudomonas aeruginosa was cultured in a 250 ml conical flask containing nutrient broth medium , It was incubated at 35-37°C using a shaking incubator at 200 rpm for 24 h. Then it was put in a centrifuge and we took the filtrate to synthesize ZnO nanoparticles according to [20] [21]with slight change 50 ml of 0.1 M zinc sulfate and 0.4 M sodium hydroxide were mixed with 50 ml of each culture filtrate of bacteria strains
, then a strong shaking and 15 minutes of heating at 40°C. To allow the nanoparticles to deposit, the flasks were microwaved for one to two minutes and then cooled for an hour.

The appearance of white sediment at the flask's bottom indicates that nanoparticles have formed.

The nanoparticles were then mixed with deionized water and centrifuged for ten minutes at 12,000 rpm. Following each centrifugation, the grains were carefully cleaned using deionized water. The grains were then gathered on a small plate, allowed to dry at room temperature, and then put in an oven at 40°C for eight hours to ensure complete drying. Powdered ZnO nanoparticles were obtained and put in tight plates for additional study, show in the figure (1) .

(a) (b)

figure (1) : (A) after adding ZnSO4.7H2O, Visual indication of synthesized ZnO nanoparticles. (B) ZnO nanoparticles completely dry

2.3.2. Characterization of Zinc oxide NPs

Several analytical techniques were used to reveal the shape, nature and chemistry of the synthesized nanoparticle . These techniques included X-ray diffraction (XRD), Ultraviolet light UV , scanning electron microscopy and EDX**.**

The size of nanoparticles and the phase of a crystalline material can both be determined using the XRD technique. Meanwhile, the structure and topography of zinc oxide nanoparticles were examined using scanning electron microscopy.

*1. **X-ray Diffraction (XRD)***

The crystalline structure of ZnO NPs was determined by using X-ray diffraction, and the sample was examined by (Cac Chemical Analysis Center. ZNO NPS were put on a glass substrate and perform at a voltage of 40 kV, a current of 8 mA), and a scan type Continuous with Cu-Kα1 radiation in a 2θ configuration. The full width at half maximum (FWHM) in the XRD was used to determine the Average crystalline size was calculated by Debye–Scherrer's equation:

$D = K\lambda/\beta\cos\theta$

In equation to determine of crystallite size (D) the K refer to Scherrer constant and λ is the X-ray wavelength and β is the full width at half maximum, while cos θ refer to Bragg's angle of reflection [22]

2.Scanning Electron Microscopy SEM and EDX

By using a scanning electron microscope equipped with an EDX spectrometer, scanning electron microscopic images of zinc oxide

nanoparticles were obtained. The images were taken at a magnification ranging from 2500 to 3500 X, and the electron beams were accelerated at 15 kV [23].

.3 ***UV-Visible Spectrophotometer***

Metal nanoparticles with sizes range 2 -100 nm are frequently estimated using light wavelengths in the 200–800 nm range [24]. Spectroscopy is a method for measuring the amount of light that a sample absorbs and scatters; this amount is called the extinction, and it is used to characterize the optical characteristics and electrical structure of nanoparticles

2.4. Evaluation of Antibacterial Activity of ZNO Nanoparticles Against Acinetobacter baumannii

The effectiveness of biosynthesis ZNO nanoparticles against *A. baumannii* was studied using an ELISA plate, based on the method mentioned by [25], with some modifications. Five bacterial isolates, numbered, were selected based on their resistance to antibiotics, biofilm production, and flow systems, by following the following steps:

1. The five bacterial isolates are activated on brain-Heart Infusion agar and incubated at 37 C° for 24 hours . Then the optical density of the bacterial suspension is measured using a spectrophotometer at a wavelength of 600nm, with the bacterial concentration of CFU/ml adjusted to become 1.5×10^{8} CFU/ml.
2. Prepare secondary zinc oxide solutions at concentrations of rang between (0 to 1.6) micrograms/ml by dissolving ZnO NPS particles in normal saline (PBS) or in sterile distilled water.
3. Prepare a 96-well ELISA plate by placing 100 microliters of bacterial culture in each well of the plate. Then, add 100 microliters of each concentration of nano zinc oxide to each well. Three replicates are made for each concentration, leaving one well as a control group in which only bacteria are placed, and another empty. Then, incubate the ELISA plate for 24 hours at 37°C .
4. This step allows the bacteria to grow and interact with the nanoparticles .
5. After the ELISA plate incubation period is over, carefully collect the medium from each well using a micropipette and transfer it to tubes for centrifugation at 5,000 rpm for 15 minutes. After that, the floating layer is used for the ELISA test .

6. compared to the control group to estimate the effect of ZnO NPS on bacterial isolates. The average optical absorbance (OD) is then calculated for the concentrations and for each replication. The percentage of effect or inhibition is then calculated according to the equation:

Inhibition percentage (%) = optical density of Control – optical density of treated sample/ optical density of control × 100%

2.5 Evaluation Antibiofilm susceptibility screening by 96microtitre well plate method :

A total of 27 *Acinetobacter baumannii* were isolated; Five strong biofilmforming isolates were selected (isolates no. 8, 19, 27, 45, and 73) . The isolates that were capable of moderate and weak biofilm producers were excluded from this study **.**

Each isolate was exposed to varying ZnO NP concentrations, ZnO nanoparticles were prepared in six concentrations: 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml.

A study of biofilm formation was performed quantitatively by using a 96well plate [26] with slight modification. Freshly cultured bacteria were inoculated in the BHI Broth and incubated at 37 °C for 24h. After 24h, the Bacterial suspensions are prepared (use five isolate). and prepare diluted ZNO nanoparticles, ZnO NPs were added at a concentration of 100 mg/ml (0.1, 0.2, 0.3,0.4 and 0.5 mg /ml) . in the sterile 96-well microplates and incubated for a further 25 hours at 36 °C. Two replicates that were not treated with ZnO NPs were used as a positive control and were also incubated for 24 hours at 37 °C without shaking. Two replicates of each bacterial suspension were kept.

After that, the plates remained inverting to dry. Optical densities (OD) were measured at 570 nm after drying, and the results were noted. After calculating the mean average OD value, the percentage of biofilm inhibition was displayed as follows :

% Percent of Biofilm inhibition = (OD570value of control− OD570 treated cells) /OD570 value of control×100

3. Results & Discussion

3.1. Acinetobacter collection and isolation

One hundred and twenty (120) specimens were collected from diffrenet clinical sources including: (burns, wounds, and urine and blood) In addition to environmental samples from the Hospital environment . Samples were taken from the (Al-Imamain Al-Kadhimain Hospital, Al-Karkh General

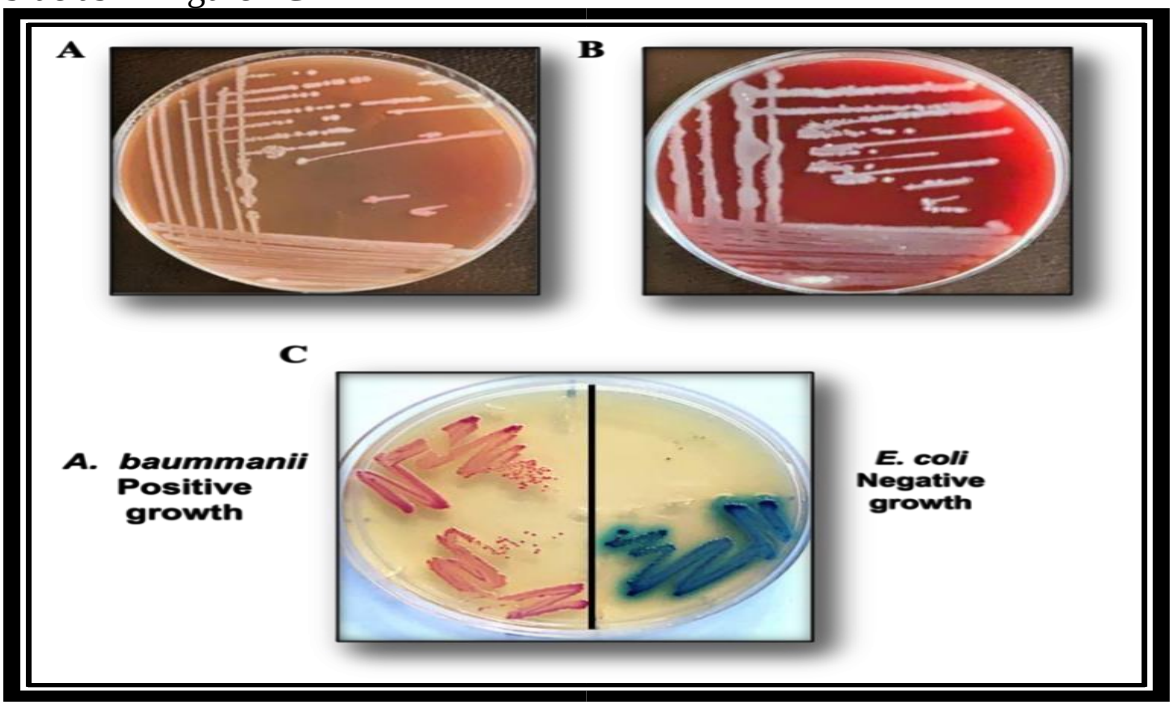
Hospital, Teaching Laboratories in Medical City) over the period from November 2024 to January 2025. All samples were collected in an aseptic condition and using a sterile container , to avoid any possible risk of contamination. All samples were cultured on blood agar and MacConkey Agar and incubated at 37 C ˚for 24h .

3.2 Identification:

3.2.1. **Morphology and Cultural Identification of *Acinetobacter* isolates :**

A.baumannii isolates were initially identified by culture on MacConkey agar, Blood agar, and CHROMTM agar: As show in Figure 2A, the *A. baumannii* colonies on MacConkey agar were tiny, smooth, and pale (nonlactose fermentative). On Blood agar, the colonies were convex, opaque, white, and lacked a hemolytic zone (nonblood hemolytic) surrounding them , However, since *Acinetobacter* does not have the required enzyme for blood hemolysis, as shown in Figure 2 B.

A. baumannii isolates were cultivated on CHROM agar, which is thought to be a selective medium for *A. baumannii* and multidrug-resistant bacteria, in order to facilitate additional identification and purification. After adding the supplement that allows the growth of only isolates of Acinetobacter, all the colonies became red. E. coli was used as a negative control, which showed blue as in Figure 2C



Figure(2) The *Acinetobacter* growth on different culture plates. The *A. baumannii* were cultured on different agar plates and incubated at 37 ºC for

24 hours. **(A)** *Acinetobacter baumannii colonies* on MacConkey agar. **(B)** *Acinetobacter baumannii colonies* on blood agar. **(C)** *Acinetobacter baumannii* and *Escherichia coli* on CHROM agar.

. 3.2.2 Microscopic Identification

According to the gram stain, the *Acinetobacter* isolates appeared gramnegative, coccobacilli bacteria and arranged as diplococci.

3.2. 3. Biochemical tests for A. baumannii

Table (1) showed the results of the biochemical testing . All isolates of *A. baumannii* gave a positive result for the catalase test. The presence of bubbles mean that the bacteria have the catalase enzyme which can break down H2O2 into H2O and O2. However , they were negative for the oxidase test due to they were unable to make cytochrome oxidase.

Moreover, the IMVIC test was used and the isolates of *A. baumannii* showed a negative result for the Indole test because the bacteria absence of the tryptophanase enzyme used to convert tryptophan into an indole ring, Similar results were shown for Methyl red and Voges-Proskauer tests. Nevertheless , a positive result was shown for the Citrate utilization test which means that the bacteria can use sodium citrate as carbon source and convert the citrate media to a blue color, as shown in Figure (3). On the other hand , Urease and motility tests showed negative results respectively because of the absence of the ability to produce a urease enzyme that breakdown urea to ammonia and does not have flagella, shown as in Table (1).

Table. 1: The biochemical tests of *A. baumannii.*

Biochemical tests	Results
Catalase	+ve
Oxidase	-ve
Indole	-ve
Methyl red (MR)	-ve
Voges Proskauer (VP)	-ve
Citrate	+ve
Urease	-ve
Motility	-ve

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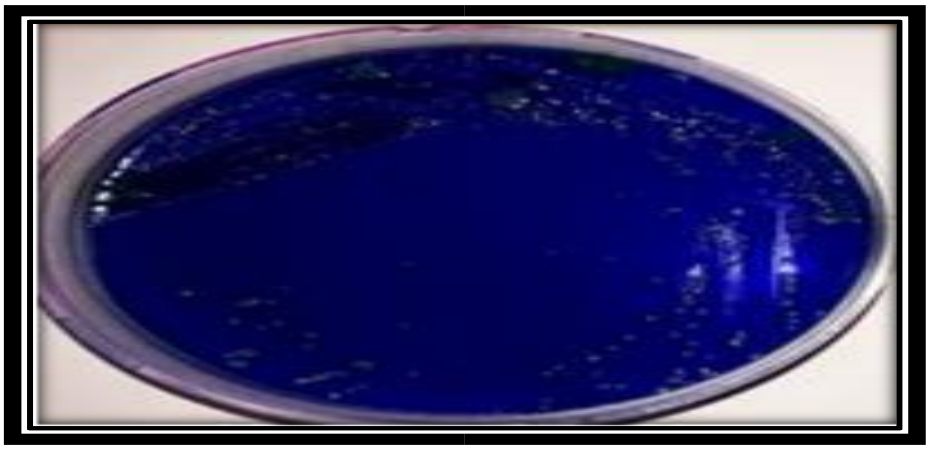


Figure (3): The growth of *A. baumannii* on citrate medium. This medium was converted to blue because *A. baumannii* used sodium citrate as the source of carbon.

3.2.4. Genetic Identification o*f A. baumannii* using 16s rRNA .

Traditional PCR was used to identify housekeeping (16s rRNA gene) which is helpful for determining *Acinetobacter baumannii* [27]. The PCR amplified sizes were 242 bp. The PCR is a more reliable method for diagnosing *A. baumannii* in clinical laboratories than Chromogenic media [28]. According to the finding, 27 (%) were diagnosed with *A. baumannii*.

3.3. ZNO NPs Biogenic Synthesis

3.3.1. Characterizations of the nanoparticles:

1. X-ray Diffraction (XRD)

Furthermore XRD analysis was formed on the dried powder in order to characterize the Zinc oxide Nanoparticles .For the X-ray diffraction analysis of the synthesis zinc oxide nanoparticles, the Cu- kα1 (1.54 [Å]) was used as the radiation source, with the diffraction angle rangin g from 0º to 100°, as shown in the figure(4) shows the several diffraction peaks at (7.10) ,(76.31),(53.20) ,(100.00),(38.78),(28.25),(36.29) and (7.46) diffraction angles Pos. [°2θ] , (22.7756), (31.8761) ,(34.5639), (36.3447),(56.6496) ,(62.9044) ,(68.1328),(95.3041) respectively.

Using the Debye-Scherrer equation, the average size of the zinc oxide nanoparticles was calculated, indicating a diffraction peak of 100 at 56.6 nm. Previous studies have found that the observed peaks vary in size and are

smaller at 2 theta values, this is due to the crystallization of bacterial metabolites, such as proteins and organic compounds, that coated the surface of the zinc oxide nanoparticles.[29]

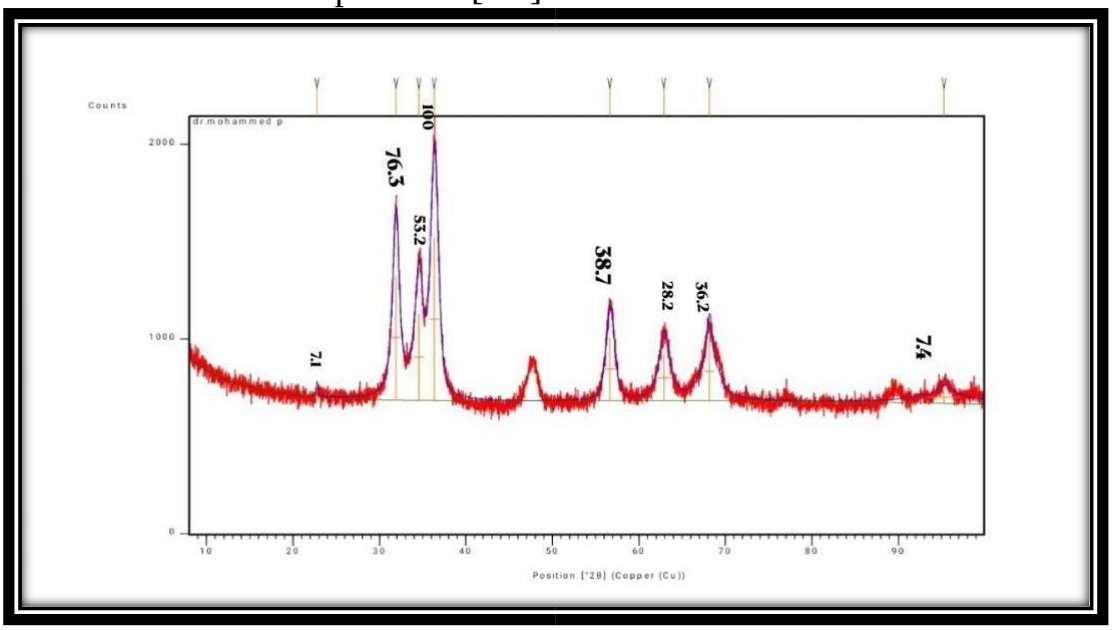


Figure (4) XRD patterns of ZnO nanoparticles

2.scanning electron microscopy (SEM) & Energy Dispersive Xray (EDX)

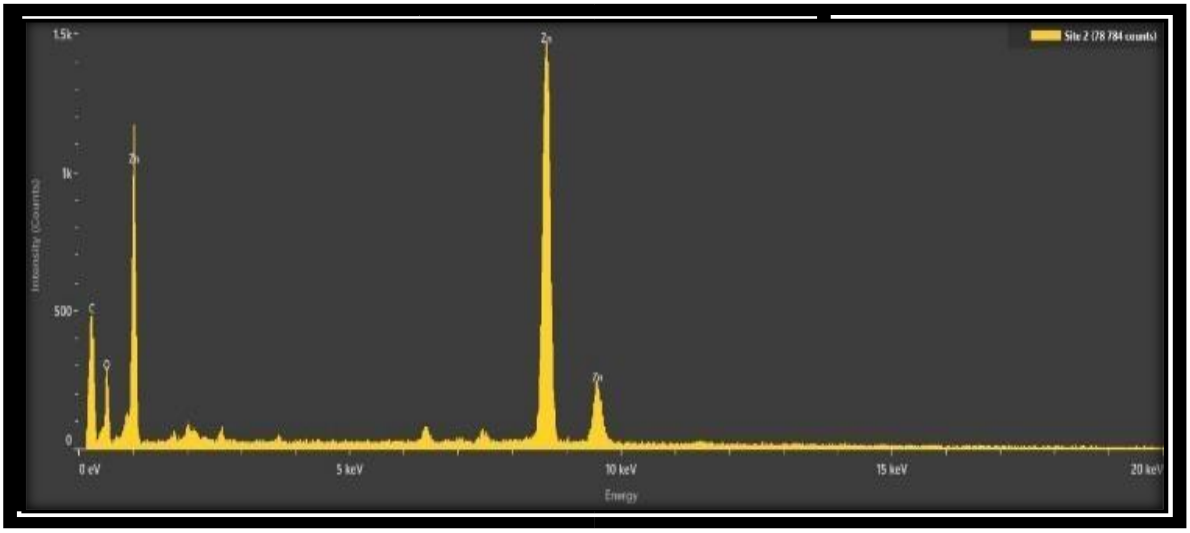
Scanning electron microscopy (SEM) is a commonly used technique to determine the shape of nanoparticles. Due to SEM images being taken from the powdered form of zinc oxide nanoparticle they may be seen as huge clusters of particles that show the size, shape, and surface morphology of green synthesized ZnO-NPs. Differences in the size and shape of green synthases zinc oxide nanoparticles have been previously documented. Due to some of the particles are capped and stabilized at a lower size while others are stabilized at a greater size, there is a great deal of variation in the form of green produced nanoparticles.

The zinc oxide NPs scanning electron microscopy (SEM) at 15,000X and 35,000X magnifications at 15 a kV is show in (Figure 5- A). It is evident from the surface scanning that the most often seen nanoparticle forms are spherical and oval. The nanoparticles are visible to be around 100 nm in size, even though SEM can't determine particle size. Using Planck's equation, the band-gap energy of ZnO-NPs was calculated:

$E = hc/\lambda$

Where h is the Planck's constant and c is speed of light , and λ is the wavelength . The band gap energy (E) for zinc oxide.
Nanoparticle is determined to be 3.326 eV. The EDX pattern in(figure 5- B) indicates that zinc has th highest elemental weight percentage found .
.

(A)



(B)

Figure (5) :(A) photomicrograph of zinc oxides Nps at 35,000X amplification. By using scaning electron microscopy ; and (B) pattern of Energy Dispersive X-ray

3. UV-Visible Spectrophotometer

The UV-visible absorption spectra of the zinc oxide Nano particles (ZnONPs) sample were detected using a UV-visible spectrophotometer in the spectral range between 200 to 800 nm. The results revealed a distinct absorption peak at 250 nm, followed by a gradual decrease in absorbance at wavelengths of 500, 600, 700 and 800 nm, these results indicate that the ZnO nanoparticles exhibit strong absorption in the UV range. consistent with their properties as a semiconductor with a dived bandgap in the 3.326ev The Sharp decrease in absorbance at wavelengths greater than 400nm indicates that the sample is transparent in the visible range, a typical optical behavior of non-aggregate ZnO nanoparticles. The absence of any Secondary absorptions as portion peaks in the Visible region is an indication of the purity of the Sample and the absence of organic or metallic impurities. show in figure (6)

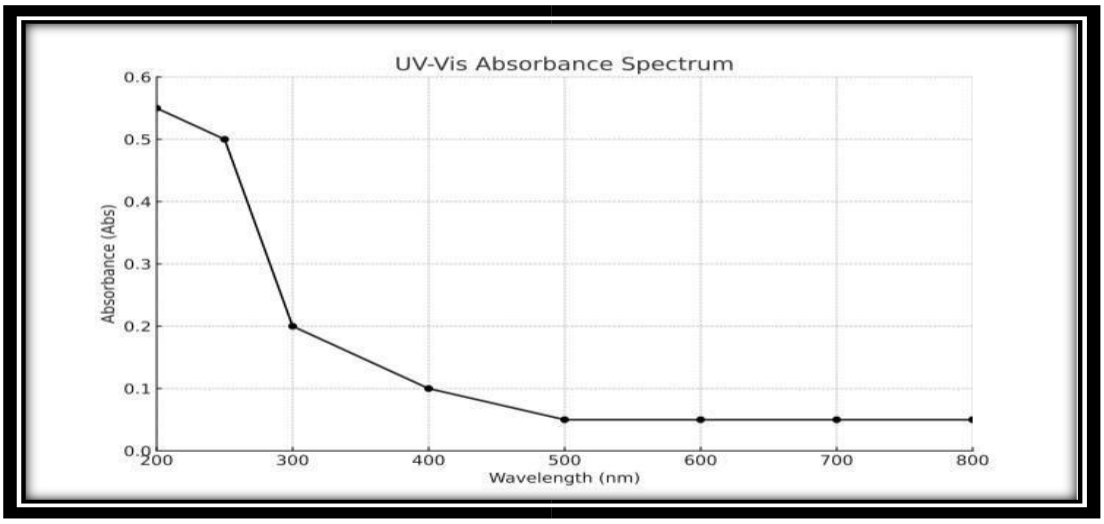


Figure (6): UV-Visible Spectrophotometer of ZnO nanoparticles.

3.4. Assessment of the Antibacterial Activity of Zinc Oxide Nanoparticles (ZnO-NPs) Using Optical Density Measurements

The antibacterial activity of various ZnO-NP concentrations (ranging from 0 to 1.6 mg/ml) was evaluated against five bacterial isolates.

The optical density (OD) It was evaluated for each concentration using a spectrophotometer as an indicator of bacterial growth. Lower OD values indicate greater inhibition of bacterial growth .The data showed a gradual decrease in optical density with increasing ZnO-NP concentrations, indicating effective inhibition of bacterial growth show in Table (2).

Table (2) shows the Antibacterial Activity of ZnO-NP (against five bacterial isolates)

Concentration mg/ml	Mean	SD	Min	Max
0	0.82	0.02	0.79	0.85
0.4	0.79	0.03	0.75	0.81
0.6	0.74	0.03	0.68	0.77
0.8	0.57	0.10	0.41	0.64
1	0.44	0.12	0.27	0.54
1.2	0.30	0.09	0.2	0.44
1.4	0.06	0.02	0.05	0.08
1.6	0.05	0	0.05	0.06

The results reveal a highly important variation between the treated and control groups (p value < 0.001), indicating a strong impact of ZnO nanoparticles on the tested isolates. This indicates that increasing the concentration of nanoparticles reduces bacterial activity.

The accompanying graph reinforces the results shown (Figure 7), showing a clear inverse relationship between ZnO-NP concentration and bacterial growth.

ZnO-NPs exhibit strong concentration-dependent antibacterial activity. These findings demonstrate that zinc oxide nanoparticle can be used as powerful antimicrobial agents in medical or industrial applications.

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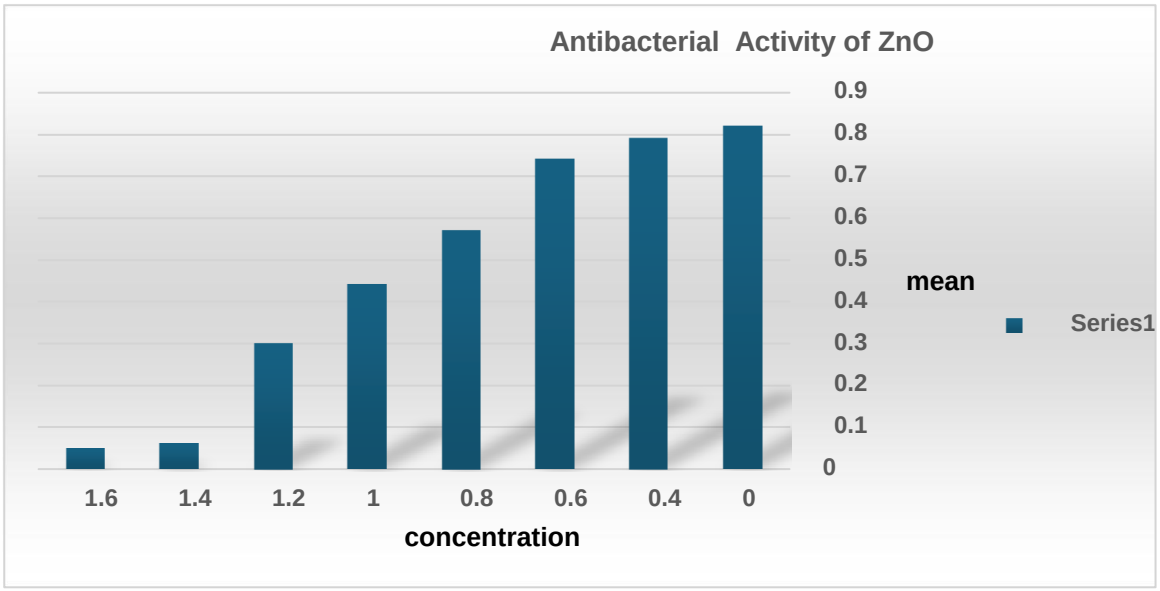

Figure (7) show Assessment the antibacterial activity of Zinc oxides NPs on *Acinetobacter baumannii*

Zinc oxides nanoparticles have demonstrated antibacterial activity against *Salmonella typhi* [30], *Vibrio cholerae*, *Shigella flexneri*, *Streptococcus mutans*, *Streptococcus pyogene*s, and bacteria specific to the skin [30, 31]. In addition, it has demonstrate antibacterial activity against *Staphylococcus aureus* that is resistant to methicillin [32]. According to a different study, Zinc oxide nanoparticles performed similarly well. antimicrobial agent based on the inhibition zone diameter against Proteus volgaris [33]. Additionally, it has been demonstrated in other research, such as this one, the ZnO Nps exhibit antibacterial activity on multidrug-resistant *Acinetobacter baumannii* isolates [34].

3.5. Evaluation of the antibiofilm effects of Zinc Oxide Nanoparticles (ZnO-NPs) Using Optical Density Measurements

Five concentrations of ZnO-NPs (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL) were evaluated against biofilm formation in five clinical isolates of *A. baumannii*. The biofilm biomass was quantified using the crystal violet staining method followed by optical density (OD) measurement, which indicates the extent of biofilm formation .

Inhibition of biofilm formation was observed depending on the concentration.. The OD values decreased progressively with increasing ZnO-NP concentration, suggesting reduced biofilm mass. The following Table (3) summarizes the results:

Biofilm formation at different ZnO-NP concentrations (first 5 isolates):

Table (3) shows the Antibiofilm Activity of ZnO-NPS

Con mg/ml	Mean	SD	Min	Max
0	0.7	0.06	0.65	0.78
0.1	0.602	0.06	0.52	0.69
0.2	0.518	0.04	0.48	0.58
0.3	0.450	0.04	0.41	0.5
0.4	0.324	0.05	0.25	0.38
0.5	0.21	0.07	0.11	0.29

The results displayed a highly important difference between the treated and control groups (p value < 0.001), indicating a strong impact of ZnO nanoparticles on the tested isolates. This indicates that increasing the concentration of nanoparticles reduces antibiofilm activity.
The data clearly show that higher ZnO-NP concentrations are associated with lower OD readings, indicating effective suppression of biofilm formation .

The accompanying graph reinforces the results shown figure (8) , showing a clear inverse relationship between ZnO-NP concentration and biofilm formation.

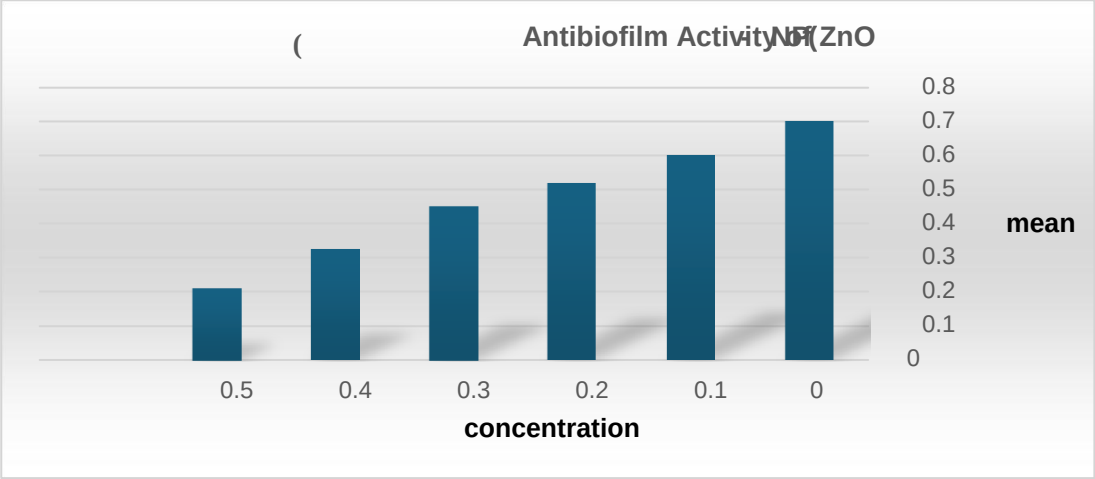

Figure (8) show the Antibiofilm Activity of ZNO Nanoparticles .

These result shows that ZnO-NPs significantly decrease biofilm formation in *A. baumannii* in a concentration-dependent method . The nanoparticles exert their effect through disruption of bacterial membranes, oxidative stress induction, and intervention with cellular processes required for biofilm development. This supports the potential use of ZnO-NPs as an alternate or adjunctive strategy in management of *Acinetobacter baumannii* infections associated with biofilms .

This highlights the promising application of ZnO-NPs in developing new approaches to combat biofilm-associated bacterial infections, particularly in antibiotic-resistant strains .

Since biofilms are an important virulence factor, the anti-biofilm activity of metal oxide nanoparticles has gained great importance in recent decades [35].

Several studies have shown that nanoparticles have an effective antibacterial and bacteriostatic effect. In a study conducted by Rahim in 2020, he revealed that zinc oxide nanoparticles are able to reduce the production of biofilms in *Pseudomonas fluorescens* bacteria.[36]

In a study by Abdel Ghaffar et al., zinc oxides nanoparticle proved their effectiveness as an antibacterial agent and reducing the activity of biofilms in Staphylococcus aureus bacteria[37]

4. Conclusion

This study demonstrated the effectiveness of zinc oxide nanoparticles as antibacterial agents and reduced biofilm formation in *Acinetobacter baumannii* .

To enhance the effectiveness of zinc oxide nanoparticles, this study recommends combining other agents, such as antibiotics, with nanoparticles to target *Acinetobacter baumannii* biofilms.

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تقييم تأثير جسيمات أكاسيد الزنك النانوية على نشاط البكتيريا وتكوين الأغشية الحيوية لبكتيريا *Acinetobacter baumannii* السريرية

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مستخلص البحث:
أجُريت هذه الدراسة في مختبرات قسم الأحياء، كلية العلوم، الجامعة المستنصرية، للفترة من (تشرين الثاني/نوفمبر **2024** إلى كانون الثاني/يناير **2025**). جُمعت (**120**) عينة من مصادر سريرية مختلفة، شملت الحروق والجروح وعينات البول والدم، بالإضافة إلى عينات بيئية من بيئة المستشفى. وشارك في الدراسة مستشفى الإمامين الكاظمية، ومستشفى الكرخ العام، ومختبرات مدينة الطب. زُرعت كل عينة على وسطي ماكونكي أجار **,blood agar**، وحُضنت طوال الليل عند درجة حرارة **37** درجة مئوية. تم تحديد **27** عزلة فقط على أنها بكتيريا ***Acinetobacter baumannii***. تم تحديد جميع هذه العزلات وتنقيتها بالزراعة على وسط كروم أجار، وإجراء الاختبارات الكيميائية الحيوية، واستخدام جين **16S rRNA**.
تم بعد ذلك تصنيع جسيمات نانوية من أكسيد الزنك حيوياً من بكتيريا الزائفة الزنجارية وكبريتات الزنك المائية(**ZnSO4.7H2O**). يتم اختزال كبريتات الزنك لتكوين جسيمات نانوية من أكسيد الزنك . تمت دراسة خصائص المادة النانوية الناتجة باستخدام أجهزة مختلفة، بما في ذلك الأشعة فوق البنفسجية، و**EDX**، و**XRD**، والمجهر الإلكتروني الماسح. أظهرت نتائج هذه الأجهزة تكوين جسيمات نانوية من أكسيد الزنك النقي بأشكال وأحجام مختلفة تتراوح بين **20** إلى **100** نانومتر.
تمت دراسة قدرة الجسيمات النانوية المصنعة على تثبيط بكتيريا ***Acinetobacter baumannii*** عند تركيزات (**4.0**، **6.0**، **8.0**، **1**، **2.1**، **4.1**، و**6.1**) ملغم/مل. وقد وجد أن فعالية جسيمات أكسيد الزنك النانوية تزداد مع زيادة التركيزات، وكانت أعلى نسبة عند تركيز **6.1** (**94**%) .
تم اختبار كفاءة المادة النانوية المُصنعّة في تثبيط بعض عوامل الضراوة ُدُرست قدرة **ZnO Nps** على تثبيط تكوين الأغشية الحيوية في بكتيريا ***Acinetobacter baumannii***. استخُدمت مجموعة من التركيزات المختلفة(**1.0**، **2.0**، **3.0**، **4.0**، و**5.0**) ملغ/مل. أظهر تركيز **4.0** و**5.0** أعلى تثبيط للأغشية الحيوية .