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Preparation, Characterization of Selenium Nanoparticles from *E.coli* and Study its Effect on Pathogenic Bacteria

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Abstract*—Bacterial infections might be regarded as one of the primary issues confronting the healthcare system at the moment. The development of nanoparticles offers an alternative to the usage of antibiotics. Sixty five isolates of *Escherichia coli* were isolated from various specimens, and used for the production of selenium nanoparticles at concentration 100 mM during 48 h, pH 8, agitation speed 160 rpm, and visualized by appearance of red color in the medium. Characterization employing Fourier transform infrared spectroscopy and UV-Vis spectroscopy showed absorption band formed at 266 nm, while, in X- Ray Diffractometer the Se-NPs peaks centered at 2θ of 27.605 , 32.092, 45.652, 56.815, 66.458 and 75.632, corresponded to the crystal planes of (100), (101), (012), (200),(022) and (210). Zeta potential demonstrate the colloidal dispersion's stability of Se-NPs with negative charge (-60mV), and Scanning Electron Microscopy showed spherically-shaped NPs with a size range between (46.71–71.91). Antimicrobial activity showed the highest inhibition zones to *Klebsiella pneumoniae* and *Staphylococcus aureus* (19.67 ± 0.58, 18.50 ± 0.87) mm respectively mm and against *Escherichia coli* was moderate inhibition (14.17 ±1.76). While the lowest zone of inhibition was observed against *P. aeruginosa* (12.00 ± 1.00) at 50 µl/ml respectively. The maximum antibiofilm activity of SeNPs was observed to be against *S. aureus*, *P. aeruginosa, K. pneumoniae* and *E. coli* MIC concentration were (89.34, 81.65, 79.81 and 80.27) % respectively, While at Sub MIC concentration were (80.81, 75.35 , 64.67 and 70.54) % respectively, So, It is an effective new antibacterial and antibiofilm agent that has applications.

Keywords—Escherichia coli, Selenium Nanoparticles, Antibacterial , Antibiofilm

I. INTRODUCTION

Green synthesis methods, or biological methods of synthesizing nanoparticles, are considered to be harmless and involve the use of biological agents such viruses, bacteria, fungi, algae, and plants. that are used for the production of nanoparticles[1].The biological synthesis method has gained popularity in recent years and is considered clean, biocompatible, non-toxic, and friendly to the environment [2,3]. When compared to nanoparticles produced by other, conventional processes, those produced by microorganisms offer extensive uses and benefits [4] Inorganic selenium production has been reported by many aerobic and anaerobic bacteria through the past ten years. (SeO_3^{2-} and/or SeO_4^{2-}) decrease accompanied by the rapid production of extracellular and intracellular SeNPs, e.g., *Escherichia coli* ATCC 35,218 [5], recombinant *E. coli* [6] , *Pseudomonas aeruginosa* ATCC 27,853 [7], Klebsiella *pneumonia* [8,9].

Selenium necessary element for both humans and animals plays an important part in the human body and helps protect it against immune-related illnesses ,through enhancing the functions of glutathione, peroxidase, selenidase, and other enzymes[10].Elemental selenium particles that are Nano sized have much greater bioactivity and biosafety characteristics, such as enhanced antioxidant, anticancer, and antibacterial activity. Thus, there is growing interest in selenium nanoparticles (Se-NPs), which may be a more effective form of selenium adding and a therapeutic agent for human health [11]. Selenium nanoparticles have shown strong biological activity and low toxicity in the medical field [12]. Because of the biomolecules' natural coating, biogenic Se NPs have greater stability and do not aggregate. The production of Se NPs is carried out by some microbes, including fungus, bacteria, and yeasts.

Because of the high surface to volume ratio and strong reactivity of Se-NPs, their capacity to penetrate bacterial biofilms and membranes, these nanoparticles have reduced bacterial viability without being highly damaging to mammalian cells [13,7]. In this study, gram-negative bacteria (*Escherichia coli)* used to bio-synthesis of selenium nanoparticles from sodium selenite, and characterized by using different techniques, and also they evaluated as antibacterial and antibiofilm against some pathogenic bacteria.

II. MATERIALS & METHODS

A. Collection of Bacteria

In this study, 130 samples were collected from several hospitals in Baghdad city/ Iraq including: Baghdad Teaching Hospital (Medical city), Ghazi Al-Hariri Hospital for Specialized Surgery ,Teaching laboratories (Medical City), , and from different sources (urine, blood, wound, stool and vaginal swabs). These including both genders of different ages (1 to 85) years and during period from (September-November/ 2023).

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B. Bacteria Isolation and Identification

Staphylococcus aureus, Pseudomonas aeruginosa, , Escherichia coli and Klebsiella pneumoniae were isolated and on MaCconkey's agar, EMB agar, Cetrimide agar and Mannitol salt agar. Identification was done according to morphological, microscopic, and biochemical examinations [14]. Also, it confirmed (65) isolates of *E.coli* bacteria and pathogenic bacteria by VITEK2 Compact System (Biomerieux, France).

C. Optimization of Produced Se NPs

Thirty isolates of *E.coli* bacteria were selected out of 65 isolates for biosynthesis of Se-NPs, and used to optimize the parameters that affect the synthesis of nanoparticles. These factors were: reaction time (hr), the concentration of Na_2SeO_3 (mM), pH value, and agitation speed (rpm).

D. Biosynthesis of Selenium Nanoparticles Using E. coli

The Se-NPs was prepared using a modified method of [15] Briefly: The bacterial suspension was prepared by cultivating single E. Coli colony in tubes containing 5 ml of Nutrient broth medium (Hi Media- India) for a 24 hr at 160 rpm overnight, then three flasks containing 100 ml of nutrient broth medium and 2 ml of filter-sterilized Sodium selenite (Na_2SeO_3) from central drug house (CDH)- New Delhi (INDIA) (con.= 25, 50 and 100) mM were inoculated with bacteria, and PH of reaction was adjusted to 8. After that, the cultures were incubation for 24- 48 hours at 160 rpm at 37°C. During this time, the solution's color change was noticed.

E. Extraction and Purification of SeNPs

To extract the Se-NPs, centrifugation was used for 10 minutes at 10,000 rpm to collect the contents of the mixture. After that, pellet washed with a 0.9% NaCl, the pellet was re-suspended in a Tris/ HCl buffer (pH 8.2) and using ultrasound disturbed for 10 minutes at 100 W[15]. Next, Millipore syringe filters (0.22 µl) were used to filter the suspensions, after that, centrifugation was used at 10,000 rpm for 30 minutes at 4 °C, the Se-NPs were extracted from the filtrates and suspended in 5 ml of ultra-pure water. The nanoparticles precipitate was washed of impurities using ethanol and water, and then dried in a hot air oven at 45 to 50 Cº [16].

F. Characterization of Selenium Nanoparticles

The UV-Visible Spectrophotometer (Spectronic-20- England) at (200–1000) nm wavelength range, Fourier Transform Infra-Red spectroscopy (-Shimadzu- Japan) at wave number range of (400–4000) cm^{-1}, Zeta potential analysis (Bruker, USA), Scanning Electron Microscope (SEM) Carl Zeiss Ultra 55 (Japan) and X- Ray Diffractometer (XRD-6000- Shimadzu Japan) were used to confirm the identity of the produced Se-NPs [17].

G. Determination of MIC Against Pathogenic Bacteria

The SeNPs was examined for the minimum inhibitory concentration against Pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, , Escherichia coli and Klebsiella pneumoniae)*. It was determined by microtiter plate according to [18] Briefly: 100 µL of SeNPs solution (con. 100- 0.195) µg/ml was added to each well that had 100 µL of Muller-Hinton broth, and about 20µL of bacterial inoculum (10^8 CFU/ml) added to it. Following by 24 hr incubation at 37 °C, then optical density at 630 nm was used to measure the inhibition of bacterial growth using ELISA Reader (Huma HS -Germany). The MIC of SeNPs that prevented visible bacterial growth was recorded.

H. Antibacterial Activity Test of SeNPs by Agar Well Diffusion Assay

According to [19] the antibacterial activity of Se-NPs was determined. The activity was tested against *Staphylococcus aureus, Pseudomonas aeruginosa, , Escherichia coli and Klebsiella pneumoniae,* in comparison to gentamicin (Bioanalye- TurkeyThe cultures were swabbed onto Muller Hinton agar plates using a sterilized cotton swab. Then 100 µl of SeNPs (con.= 50, 25, 12.5) µg/ml were added to the plate's wells (8 mm) using a micro-pipette in addition to disc of antibiotic as standard control for pathogenic bacteria. At 37°C, the plates were incubated for 24 hours. The sizes of the inhibition zones were recorded and measured in millimeters.

I. Biofilm Formation

The biofilm formation of pathogenic bacteria was investigated using according to [20] with modification. About 100µL of Brain heart infusion broth supplemented with 2% sucrose was added to each well of a microtiter plate. After that, the wells were filled with 100µL of pathogenic bacteria suspension (10^8 CFU/ml), and they were then incubated for 48 hours at 37°C. Moreover, to eliminate free-floating bacterial strains, 200 µL of phosphate-buffered saline (pH=7.2) was used to rinse the wells three times.. The attachment to the plate was examined by staining with 0.1% crystal violet solution. At 570 nm, the OD value was measured using ELISA reader after the biofilm mass had been de-stained for 20 minutes using 95% ethanol [21].

J. Antibiofilm of SeNps

The antibiofilm activity of SeNPs was investigated according to [22]. with modification. About 100µL of BHI broth supplemented with 2% sucrose was added to each well of plate. The wells were filled with 10µL of pathogenic suspension (10^8 CFU/ml) and 100µl of SeNPs at (MIC and sub MIC) concentration , and they were then incubated for 48 hr at 37°C. The wells were rinsed three times with 200 µL of phosphate-buffered saline (PH= 7.2) to eliminate free-floating bacterial strains. Then staining with 0.1% crystal violet solution and de-stained for 20 minutes using 95% ethanol, the test was performed in triplicate. The optical density value was measured at 570 nm using an ELISA reader, and the inhibition % was calculated using the following equation:

$$\text{Inhibition } \% = \frac{OD\ of\ control - OD\ of\ treatment}{OD\ of\ control} \times 100\%$$

K. Analytical Statistics

In the statistical analysis the SPss program was applied and statistical significance was assessed using t tests .P value was defined as less than 0.05.The data collected were

expressed as mean ± standard deviation (SD). The experiment was performed in triplicate.

III. Results and Discussion

A. Bacterial Isolates

About 65 isolates of *Escherichia coli* were isolated and identified by using light compound microscope as Gram-negative bacteria, bacilli, rod-shaped cell [23] also fermented lactose on MaCconkey's agar (fig.1.a) and colonies exhibited a green metallic shine on Eosin Methylene Blue (fig.1.d). Accreditation was used to carry out the isolation and diagnosis process of [24.25].

Twenty isolates of tested pathogenic were isolated, *S.aureus* colonies appeared with yellow color from fermenting Mannitol when cultured on Mannitol salt agar(fig.1.b), while *P.aeruginosa* on Cetrimide agar gave green color (fig.1.e) , and *K. pneumoniae* colonies were fermented lactose on MaCconkey's agar (fig.1.c), and this similar to the typical features mentioned in previous investigations of [26]. VITEK2 Compact System 2 confirm the identification of Gram-positive and Gram-negative isolates, The isolates that were most resistant to antibiotics were selected for application.

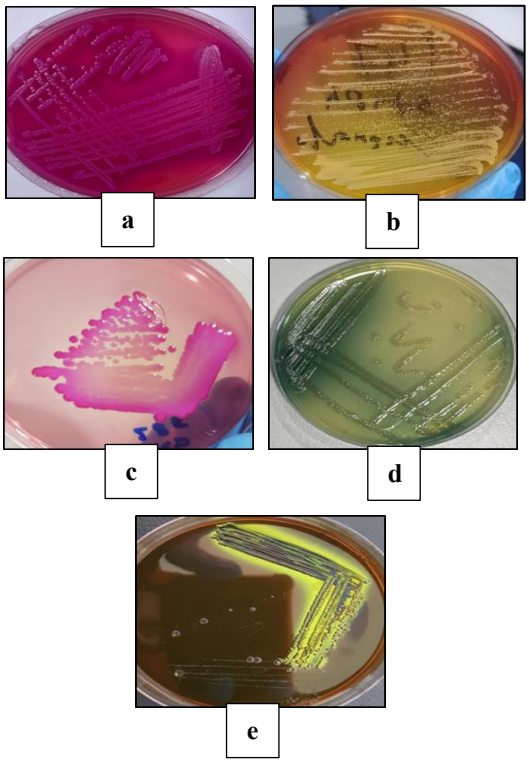


Fig. 1. a) *E.coli* on macConky agar. b) *S.aureus* on mannitol salt agar. c) *K.pneumoniae* on macconky agar. d) *E.coli* on EMB. e) *P. earugenosa* on Cetrimide agar.

B. Optimization and production of Se-NPs

The change in color from yellow to red indicates the production of SeNPs as shown in figure (2), which showed how Se was reduced to Se0[22]. Significant dark red color was not seen at low Se values (25 mM), but it gradually became visible at higher concentrations (50 and 100 mM). when incubation was continued to 24- 48 h, and when pH was 8 and agitation speed was 160 rpm (Table 1). The total Se^0 content in the SeNPs steadily increased with high concentrations, and supported by findings of [27]. Absorbance of SeNPs results Figure (2.d) indicated that isolate number 10 (RN-10) was the best isolate for nano production through the intensity of the color formed and the high degree of absorbency. Therefore, it was chosen in further experiments.

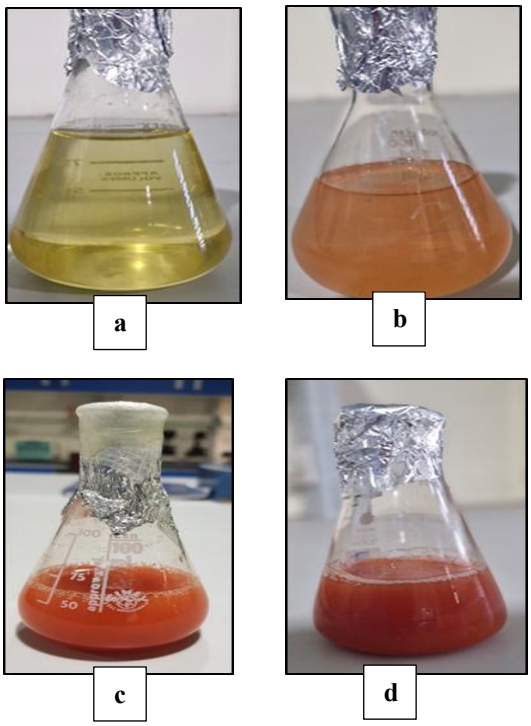


Fig. 2. a) control without Na_2SeO_3. b) Na_2SeO_3 25 Mm. c) Na_2SeO_3 50 Mm. d) Na_2SeO_3) 100M.

Table 1. Values of Se-NPs produced by *E.coli* isolates according to growth conditions and the absorbency

No.	24 hr.			48 hr.			Absorbance (266 nm)
	Con.25 mM	Con.50 mM	Con.100 mM	Con.25 mM	Con.50 mM	Con.100 mM	
1	++*	++	+++	++	+++	+++	0.99
2	+	++	+++	+	+++	+++	0.68
3	+	+	+++	++	++	+++	0.7
4	+	+	+	+	+	+	0.31
5	+	++	++	+	++	+++	0.63
6	+	+	++	+	++	++	0.43
7	+	++	+++	++	+++	+++	0.89
8	+	+	++	++	++	+++	1.1
9	+	+	++	++	+++	+++	1.2
10	+	++	+++	++	+++	+++	1.3
11	+	+	+	+	+	+	0.39
12	+	+	++	+	+	++	0.76
13	+	+	+	+	+	+	0.31
14	+	++	++	++	+++	+++	0.71
15	+	+	++	++	+++	+++	0.74
16	+	++	++	++	++	++	0.42
17	+	++	+++	++	+++	+++	1.09
18	+	++	++	+	++	+++	0.73
19	+	+	++	++	++	++	0.65

20	+	+	+	++	++	++	0.63
21	+	+	+	+	++	++	0.39
22	+	+	++	+	++	++	0.4
23	+	++	+++	+	++	+++	0.7
24	+	+	+	++	++	++	0.39
25	+	+	+	++	++	++	0.37
26	+	++	++	+	++	++	0.4
27	+	+	+	+	+	+	0.23
28	+	+	++	+	++	++	0.42
29	+	++	+++	+	++	+++	0.74
30	+	+	+	+	+	+	0.27

IV. CHARACTERIZATION OF SENPS

A. UV-vis Spectrum Analysis

The UV-Vis absorption spectra showed a peak formed at around 266 nm, which is attributed to Se-NPs. Characterization of Se-NPs' absorption peak is shown in Figure 3. The present work was in accordance with [12,28].

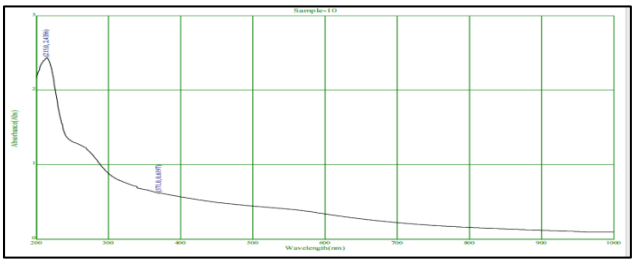


Fig. 3. Absorbance peak of selenium nanoparticles by UV-Vis spectroscopy

B. FTIR Analysis

The FTIR spectra of the selenium nanoparticles are shown in Figure (4) and the results presented the peak value around 3330 cm-¹ may be due to the presence OH in carbohydrates, proteins and phenol. The absorption peak around 2359 cm-¹ can be the peak of C-C conjugated and 2105 cm-¹ can be the peak of C-C unsaturated compound .The peak at 1635 cm-¹ represents the C-O group, and The peak at 490 cm-¹ related to metal–carbon stretch. Spectra indicated the presence of many functional groups, which could be in charge of the stability and reduction of selenium nanoparticles [12]. The current of this study was to agree with [29,30].

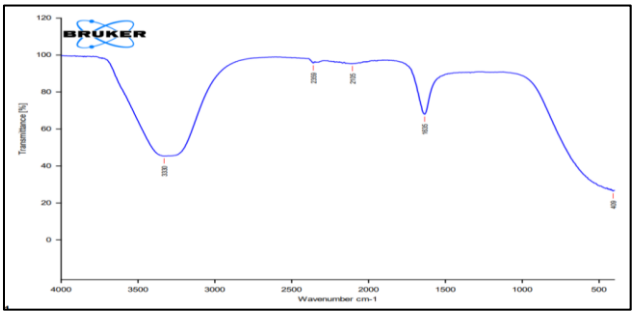


Fig. 4. FTIR spectra for selenium nanoparticles

C. XRD Analysis

The X-ray diffraction was used to characterize the crystal structure and composition of the produced nanoparticles, which gave the results in Figure (5). One noticed the peaks were sharp and narrow, the selenium peaks centered at 2θ of 27.605, 32.092, 45.652, 56.815, 66.458 and 75.632, corresponded to the crystal planes of (100), (101), (012), (200),(022) and (210) (JCDPS card no. 01-085-0567) standard. The Se-NPs having hexagonal structure were successfully formed, and the lattice constants [31].

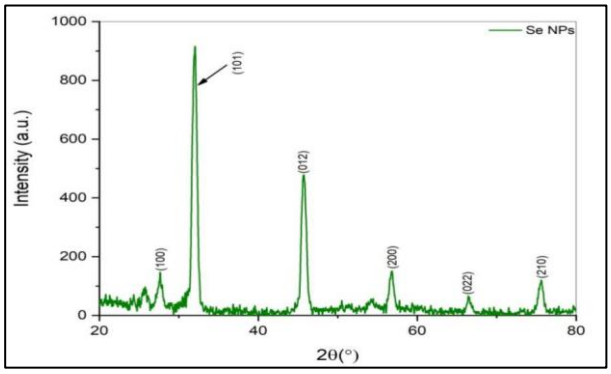


Fig. 5. XRD analysis of selenium nanoparticles

D. Zeta Potential

It is used to measure colloidal dispersion's exceptional stability and the nanoparticles' capacity to attach to cell membranes. Results in Figure 6 showed a peak (-60mV) within the stability region (-35 to +35) with negative charge. A positively charged component of cell membranes is suggested to have a good adhesion potential by a negative charge; similar results were also reported by [32].

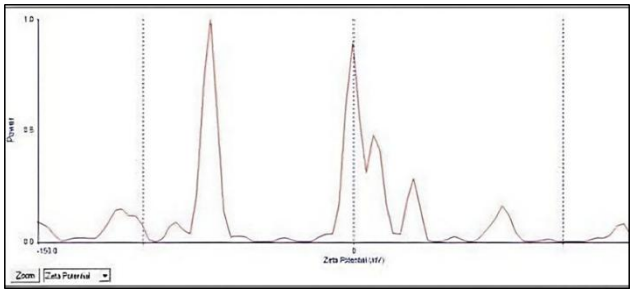
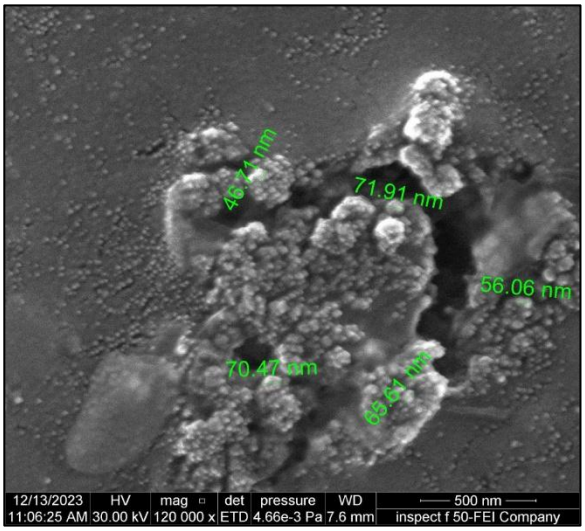


Fig. 6. Zeta potential analysis of selenium nanoparticles

V. SCANNING ELECTRON MICROSCOPY ANALYSIS

It was used to approve the size and shape of the biogenic selenium nanoparticles, as well as the evenly distributed, Results showed spherically-shaped selenium nanoparticles made by *E. coli* with a size range between (46.71 – 71.91) figure (7). This result is agreements with [33].



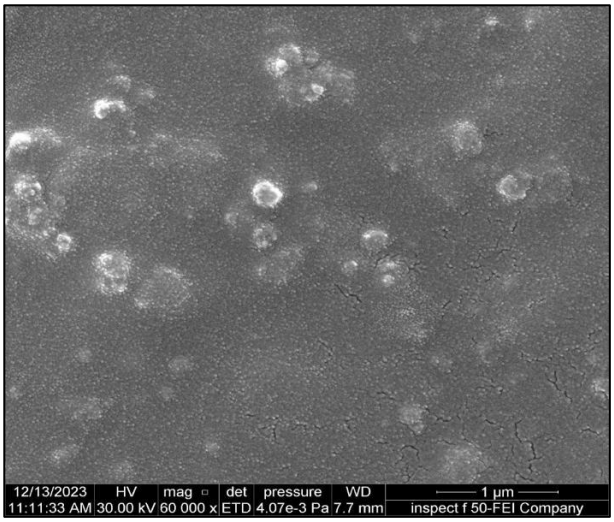


Fig. 7. SEM picture of Se-NPs nanoparticles synthesized by *E. coli.*

A. MIC determination

The MIC (minimum inhibitory concentration) of SeNPs against pathogenic bacterial were estimated by microtiter plate method. The MIC of SeNPs against *S. aureus , P. aeruginosa* and *E. coli* was 25 µg/mL while MIC of SeNPs against *K. pneumoniae* was 12.5 µg/ml. From the results gained and summarized in Table (2), The study showed that *K. pneumonia* bacteria were more sensitive than the other isolates which inhibited at 12.5 µg/mL ,However *S. aureus , P. aeruginosa* and *E. coli* bacteria were more resistance to SeNPs at that concentration . The electrostatic interactions that cause Bio-SeNPs to adhere to the bacterial cell wall and cause bacterial death may be the cause of the lower MIC values of Bio-Se-NPs. As the Se-NPs synthesized using *E.coli* has exhibited strong antimicrobial activity against pathogenic bacteria .These results are similar to a study conducted by Researchers [22].

Table 2. MIC of Se NPs against pathogenic bacteria

Pathogenic bacteria	Minimum inhibition concentration (MIC)
Staphylococcus aureus	25 µg/ml
Pseudomonas aeruginosa	25 µg/ml
Klebsiella pneumoniae	12.5 µg/ml
Escherichia coli	25µg/ml

VI. ANTIBACTERIAL ACTIVITY OF SE-NPS

As shown in Figures (8), SeNPs antibacterial activity was determined at concentrations of 50, 25, and 12.5 µg/ml by the well diffusion agar assay. As-synthesized SeNPs showed good preliminary antibacterial action against tested pathogenic bacterial (Table 3). The strong antibacterial activity of SeNPs (50 µg/ ml) was observed against *K. pneumoniae, and S.aureus* which zone of inhibitions were observed in the range of (19.67 ± 0.58, 18.50 ± 0.87) mm respectively, and against *E.coli* was moderate inhibition (14.17 ±1.76). While the lowest zone of inhibition was observed against *P. aeruginosa* (12.00 ± 1.00). When the SeNPs concentrations were 25 µg/ ml, the diameter of inhibition zones decreased to (10.67 ±1.53, 7.83 ± 0.29, 10.00 ± 2.65 and 9.67 ±1.53) mm against *S. aureus*, *P. aeruginosa, Klebsiella pneumonia* and *E. coli*, respectively, were on the other hand, SeNPs at 12.5 µg/ml had no antibacterial effect on tested strains. SeNPs showed statistically significant antibacterial action at 50 µg/ml. (***P value <0.05) According to the results, Gentamicin were used negative control to compare with inhibitory activity of SeNPs against pathogenic bacteria that were resistant to that antibiotic. The antibacterial effects of SeNPs on bacteria have been shown in a variety of studies, the primary cause of differences in the results of several investigations is the variation in the size of nanoparticles and the kind of bacteria employed. Particle size and concentration are two of the most significant variables influencing the antibacterial capabilities of nanoparticles. Smaller nanoparticles were thought to have produced more reactive oxygen species (ROS) than bigger surface area to volume ratios inside or outside of the cells. Increasing the surface to volume ratio of nano materials enhances their antibacterial properties. As a result, could show many antibacterial activity mechanisms, including the antibacterial physical properties of the nanoparticle connected to membrane disruption or cell wall penetration, as well as the release of antibacterial metal ions from the particle surface. Therefore the antimicrobial effect of SeNPs was more evident at concentration (50 µg/ml) ,and this results is agreements with [34,35,36,16,11].

A. Biofilm formation for pathogenic bacteria

The biofilm production assay was used to determine which bacterial strains were the most capable of forming biofilms. In table (4) the obtained results revealed that *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* isolates formed biofilm with strong attached. The identification of bacteria strains that produce biofilm, which are frequently resistant to the available antibiotics, has been confirmed by the VITEK2 Compact System 2.

Table 3: Antibacterial effect of different concentrations of SeNPs against pathogenic bacteria

Pathogenic bacteria	Inhibition zone mm in 50 µg/ml Mean ±SD	Inhibition zone mm in 25 µg/ml Mean ±SD
Staphylococcus aureus	18.50 ± 0.87	10.67 ±1.53
Pseudomona aeruginosa	12.00 ± 1.00	7.83 ± 0.29
Klebsiella pneumoniae	19.67 ± 0.58	10.00 ± 2.65
Escherichia coli	14.17 ±1.76	9.67 ±1.53

In this results Microorganisms adhere to surfaces and release extracellular polysaccharides, which cause a biofilm to grow. Because biofilm-associated microorganisms are becoming more resistant to antibiotics and have the potential to infect people with indwelling medical devices, biofilms represent a significant threat to public health, and these findings are consistent with [37,38].

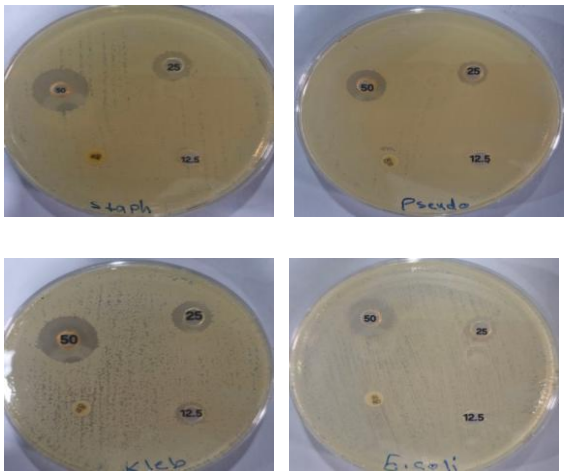


Fig. 8. Antibacterial effect of (50, 25,12.5) µg/ml of Se-NPs against pathogenic bacteria

Table. 4. Biofilm formation by the Microtiter plate method for pathogenic bacteria

Bacterial isolates	Biofilm formation after 48 h
Staphylococcus aureus	strong
Pseudomonas aeruginosa	strong
Klebsiella pneumonia	strong
Escherichia coli	strong

B. Antibiofilm effect of Se-Nps

The obtained results demonstrated that the antibiofilm concentration of SeNPs against *S.aureus, P. aeruginosa, K.pneumoniae* and *E.coli* ,at MIC and sub MIC was recorded (Table 5), (fig 10). The antibiofilm against *S. aureus, P.aeruginosa*, *K. pneumoniae* and *E. coli* at MIC concentration were (89.34, 81.65, 79.81 and 80.27) % respectively, while at sub MIC concentration were (80.81, 75.35 , 64.67 and 70.54) % respectively. Through these results, The biofilm forming ability of all tested strains changed, Where all strains decreased their productivity of the biofilm . biogenic SeNPs show activity against pathogenic bacteria prevent biofilm Which depends in its effect on concentration , where it was found that SeNPs have inhibitory action against the formation of biofilms at MIC greater than Sub MIC concentration. Biogenic SeNPs exhibit significant inhibition through their capacity to prevent biofilm formation in its early phases and even dissolve them in their mature stages. where After the biofilm is destroyed, the bacteria dies due to the breakdown of the glycocalyx, which is the glycoprotein and glycolipid coating. This study was consistent with [39,40,22].

Table 5. Antibiofilm for pathogenic bacteria at MIC and sub MIC

Bacterial isolates	**Inhibition of biofilm at MIC%**	**Inhibition of biofilm at Sub MIC %**
Staphylococcus aureus	89.34 %	80.81 %
Pseudomonas aeruginosa	81.65%	75.35 %
Klebsiella pneumonia	79.18 %	64.67 %
Escherichia coli	80.27 %	70.54 %

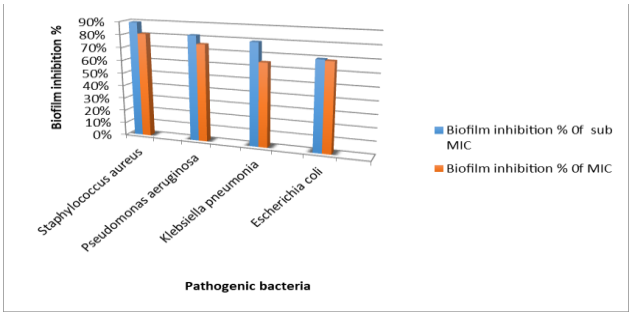


Fig. 10. Biofilm inhibition percentage of sub-MIC and MIC

VII. CONCLUSION

According to the results, selenium NPs that were biologically produced using E. coli bacteria showed strong antibacterial

properties against pathogenic bacteria. The produced nanoparticles' favorable structural characteristics were demonstrated by a variety of analysis techniques. In addition, The results confirmed the prediction that, when generated in optimal conditions, NPs would prevent pathogenic bacteria from forming biofilms. Selenium nanoparticles have appropriate antibacterial and antibiofilm characteristics against pathogenic bacteria, making them useful in combating against microbial diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

No funding was received for conducting this study

ETHICAL CONSIDERATIONS

The research was appraised and approved by the Research Ethics Committee of Mustansiriyah University\ College of Science- Dep. Of Biology, under code number, Ref: BCSMU\0923\ 0005B approval ID.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by **[Rasha muhsin yasir]**, [**Neihaya Hekmet Zaki]**. The first draft of the manuscript was written by [**Rasha muhsin yasir**] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

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