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Antimicrobial effect of biosynthesis of selenium nanoparticles by Pseudomonas aeruginosa in bacteria isolated from wounds and burns

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Abstract: background: In recent decades, the rise of antibiotic resistance as critical global health challenges, necessitating innovative approaches to address these pressing issues. The Center of Disease Control (CDC) estimates that antibiotic resistance is responsible for nearly 5 million deaths in 2019 Objective: This study aims to biosynthesise selenium nanoparticles and the possibility of using as antimicrobal. Methods: Samples were collected from Al-Diwaniyah Teaching Hospital in Al-Diwaniyah Governorate - Iraq, period from April 1, 2023 to June 1, 2023. Bacterial samples were collected from wounds and burns, then they were diagnosed using (Vitek-2 compact system-Biomerieux-France), then all the collected bacterial species were tested to produce selenium nanoparticles and the best bacteria capable of producing them were selected. The synthesis process involved incubation, centrifugation, and purification. The antimicrobal activity of SeNPs was evaluated. Results: *Pseudomonas aeruginosa* was the predominant pathogen accounting for 30%, followed by *Bacillus* (24%), *Staphylococcus* (16%), *Escherichia coli* (8%), and *Candida albicans* (12%). Mixed infections are found in 10%. Statistical analysis showed (chi-square = 40.32, $p < 0.0001$). The *bacilli* showed high sensitivity to ciprofloxacin (Cip) with a mean diameter of 28 ± 1.02, while amoxicillin (AX), ceftoxime (CTX), doxycycline (DO), and carbenicillin (PY) had no inhibitory effect (0 ± 0). *Staphylococci* showed sensitivity to ciprofloxacin (18 ± 0.64), amoxicillin (13 ± 0.22), cefatoxime (12 ± 0.4), doxycycline (12 ± 0.4), nitrofuranthone (THAT) (11 ± 1), and neomycin (17.2 ± 1.1). *Escherichia coli* showed sensitivity to ciprofloxacin (28 ± 1.02) and doxycycline (19 ± 1.4), while *Candida albicans* showed resistance to all antibiotics tested. Antimicrobial activity was measured using concentrations of 125, 250, 500, and 1000 µg/mL. Results show a concentration-dependent increase in inhibitory effects. Antibiofilm activity ranges from 2 to 1048 µg/mL.Discussion: Lower concentrations (125 µg/ml), minimal inhibition was observed, but as the concentration increased, a clear trend of enhanced inhibition emerged. Notably, at 250 µg/ml, significant growth inhibition was evident, with a concentration-dependent response. The highest concentration (1000 µg/ml) exhibited the most substantial inhibitory effects. The absence of inhibition in the negative control confirms the specificity of nano selenium's impact.conclusion. : The results demonstrate concentration-dependent inhibitory effects of nano selenium on various bacterial species.

Keywords: Selenium nanoparticles, Pseudomonas aeruginosa, antimicrobial activity, Biosynthesis nanoparticles, antibiotic resistance, Nanomaterial synthesis

1. INTRODUCTION

In the twenty-first century, antimicrobial resistance has assumed a paramount role, posing a significant threat to global public health. Failure to promptly address antimicrobial resistance is projected to result in an alarming toll of 10 million deaths annually and up to $100 trillion in global economic losses by 2050 [1]. The

biochemical resistance mechanisms used by bacteria include the following: antibiotic inactivation, target modification, altered permeability, and “bypass” of metabolic pathway [2].

Nanoscience and nanotechnology represent a modern and evolving field in the world of science and technology, focusing on understanding and controlling the unique properties of materials at the nanometer level (about 1-100 nanometers) , this field is central to innovation and development in various fields, from medicine to electronics and environmental sciences [3]. Nanoparticles have pivotal mechanism contributing to microbicidal activity involves the generation of free radicals by nanoparticles. These radicals have the ability to damage cell membranes, making them porous and eventually leading to cell death. Metal nanoparticles show a tendency to interact with sulfur- and phosphorus-containing biomaterials found in bacterial cells, such as DNA bases. Metal nanoparticles can act on these soft bases, leading to DNA damage and subsequent cell death [4]. Selenium, a trace element found in soil, water, and certain foods, has gained considerable attention in recent years because of its well-established antibacterial properties [5]. Selenium nanoparticles can be synthesized by using microorganisms such as bacteria, fungi, and algae [6].

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium. It is a member of the genus Pseudomonas, which is part of the family Pseudomonadaceae. P. aeruginosa is a ubiquitous bacterium that can be found in soil, water, and on plants. It is also a common inhabitant of the human body, where it can be found in the respiratory tract, gastrointestinal tract, and genitourinary tract [7]. Bio-synthesis of nanoparticles by *P. aeruginosa* is a process by which the bacteria produce nanoparticles from organic precursors. The bacteria use a variety of enzymes to convert the precursors into nanoparticles, which can then be used for a variety of purposes [8]. The bio-synthesis of nanoparticles by *P. aeruginosa* is a promising new technology with a variety of potential applications. The bacteria are able to produce nanoparticles with a variety of properties, which makes them a versatile platform for a variety of applications [9].This study aims to biosynthesise selenium nanoparticles and the possibility of using them as antimicrobal.

2. MATERIALS AND METHODOLOGY

2.1. Sample collection

Between April 1, 2023, and June 1, 2023, samples were taken from Al-Diwaniyah Teaching Hospital in the Al-Diwaniyah Governorate in Iraq. After bacterial samples from burns and wounds were gathered, the best bacteria were chosen to make selenium nanoparticles by testing each sample for their ability to do so.

2.2. Synthesis of nanoparticles

All samples were cultured and diagnosed, dilutions were prepared by mixing 10 ml of sample with 100 ml of normal salt, from which a bacterial suspension was prepared for proliferation in tubes. The tube was prepared and placed in a precipitator for 24 h at 37°C. Next, the tube was placed in the centrifuge at 10,000 rpm for 10 minutes. 10 ml of bacterial filtrate was mixed with 5 mM selenium salts. Both tubes (negative control and filter mixed with selenium salts) were placed in a precipitator at 30°C for 24 h. A color change was observed

from yellow to walnut, indicating the formation of nanomaterials. Purification of the nanomaterials was performed using a centrifuge at 10,000 rpm for 5 min[10].

2.3. SeNps antimicrobial activity

This experiment was performed according to [11]. The culture media were created according to the manufacturer's specifications. After sterilization. The medium was cooled to 45-50°C and then placed on plates to a depth of approximately 4 mm. Plates were kept at 4°C until used because the agar had solidified throughout the process.

When preparing the inoculum, culture colonies (18-24 h) of the isolates were transferred to a 5 ml tube of normal saline to generate a density of 1.5*108 cells per culture ml equivalent to 0.5 McFarland standard solution measured at 600 nm which were then used to prepare the inoculum.

Plate inoculation was performed by soaking a sterile swab. Next, Mueller-Hinton agar plates were inoculated by running the swab along the sterile agar surface (rotated by 60°). This method was repeated. Finally, the swab was moved around the edge of the agar surface to ensure even distribution of bacteria. The plates were incubated at 37°C for 30 minutes, for 24 hours.

Screened against a variety of multidrug-resistant bacterial strains identified from clinical settings: *E.coli*, *Staphylococcus aureus* , and *P. aeruginosa* was among the microorganisms identified (as it is the most common bacterial species in the current study). This study used a microdilution technique to determine the minimum inhibitory concentration (MIC), which was modified to comply with the Clinical and Laboratory Standards Institute (CLSI) reference procedure [12].

The final concentration of cells was adjusted using Mueller-Hinton broth in a spectrophotometer at 625 nm with an optical density of 0.08–0.1 to achieve a concentration of 5105 CFU/ml using a spectrophotometer at 625 nm with an optical density of 0.08–0.1.

2.4. Statistical analysis

All data were analyzed using SPSS software (V.28 Inc., Chicago, USA). and Kolmogorov-Smirnov test of distribution of variables. Usually, the numerical variables distributed between the treated groups were compared using a two-way ANOVA test (univariate and multivariate analysis so that the variance was obtained for the least significant difference (LSD) , and all data were expressed as a standard deviation (mean ± SD) and the significance of the differences was discovered when... $p < 0.05$ This experiment was conducted according to [13] .

3. RESULTS

3.1. Bacterial Diagnosis

The results reveal the prevalence and distribution of various germ types within the examined samples. *Pseudomonas aeruginosa* emerged as the most frequently diagnosed germ, constituting 30% of the total isolates, followed closely by *Bacilli* at 24%. *Staphylococcus* accounted for 16%, while *Escherichia coli* and *Candida albicans* represented 8% and 12%, respectively. Mixed infections were identified in 10% of the isolates. In total, 150 isolates were analyzed, encompassing a diverse range of germ types. The statistical analysis, with a Chi-square value of 40.32 and an impressively low probability value (<0.0001), as shown in figure 1 .

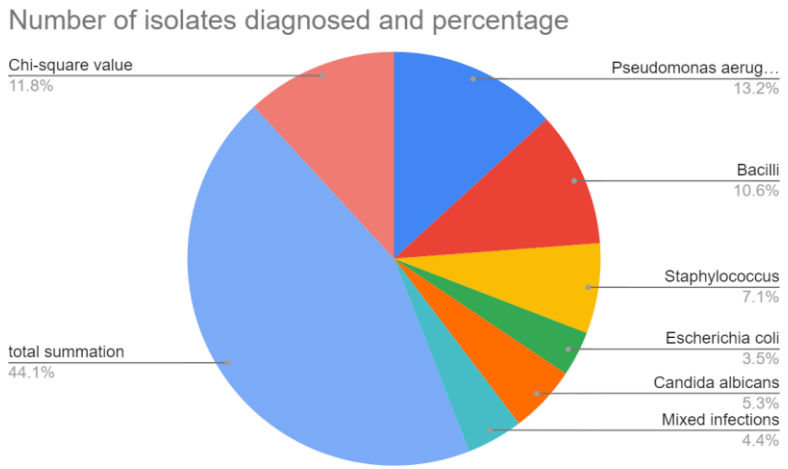

Figure 1 percentage distribution of bacteria isolated in current study.

3.2. Antibiotic Sensitivity test

The table 2 presents the sensitivity of various identified bacterial species to a range of antibiotics. For *Bacilli*, Ciprofloxacin (Cip) displayed the highest inhibitory effect with an average diameter of 28±1.02, whereas Amoxicillin (AX), Cefatoxime (CTX), Doxycycline (DO), and Carbenicillin (PY) showed no inhibitory effect (0±0). *Staphylococcus* exhibited sensitivity to Ciprofloxacin (18±0.64), Amoxicillin (13±0.22), Cefatoxime (12±0.4), Doxycycline (12±0.4), Nitrofuranthone (THAT) (11±1), and Neomycin (17.2±1.1). *Escherichia coli* was only sensitive to Ciprofloxacin (28±1.02) and Doxycycline (19±1.4), while *Candida albicans* showed resistance to all antibiotics tested. The LSD value of the least significant difference was calculated as 0.911. These values, representing the diameter of growth inhibition (average ± standard deviation) for five isolates of each bacterial type, highlight variations in antibiotic sensitivity across different bacterial species.

Table 2 Sensitivity of the identified micoorgansim species to a number of antibiotics

Type of antibiotic	**The type of bacteria tested**			
	Bacilli	***Staph. spp***	***E. coli***	***C. albicans***
Ciprofloxacin	28±1.02	18±0.64	0±0	---
Amoxicillin	0±0	13±0.22	0±0	----
Cefatoxime	0±0	12±0.4	0±0	----
Doxocycline	19±1.4	12±0.4	0±0	----
	0±0	0±0	0±0	----
Nitrofuranthone	12±0	11±1	0±0	----
Carbenicillin	0±0	9±0.2	0±0	----
Neomycin	-----	----	0±0	17.2±1.1
LSD value of least significant difference	0.911			

Values represent the diameter of growth inhibition (average ± standard deviation) for five isolates of each bacterial type.

3.3. Antimicrobial activity of SeNPs

The presented results in Table 3 and figure 2 reveal the sensitivity of identified bacterial species to varying concentrations of nano selenium. The investigation involved *Bacilli*, *Staphylococcus*, *Escherichia coli*, and *Candida albicans*, and the inhibitory effects were measured at different nanoscale selenium concentrations (125, 250, 500, and 1000 µg/ml). At the lowest concentration (125 µg/ml), negligible inhibition was observed across all tested bacterial types. However, as the concentration of nano selenium increased, a clear trend of enhanced inhibitory effects emerged. For instance, at 250 µg/ml, noticeable growth inhibition was observed, with values ranging from 10.1±0.5 to 15±1.1, indicating a concentration-dependent response. Further elevating the concentration to 500 µg/ml resulted in increased inhibitory effects, as reflected in the values of 13.8±0.14 to 20.1±0.56. The highest concentration of nano selenium (1000 µg/ml) exhibited the most significant inhibitory effects, with values ranging from 18.2±0.42 to 26.6±0.4. The negative control, represented by distilled water, showed no inhibitory effect, reinforcing that the observed effects were attributed to the presence of nano selenium. The LSD value of least significant difference (0.814) indicates the variability among different concentrations and underscores the statistical significance of the observed inhibitory effects.

Table 3 : Sensitivity of the identified bacterial species to different concentrations of nano selenium

Nanoscale selenium concentrations (µg/ml)	The type of bacteria tested			
	Bacilli	***Staphylococcus***	***Escherichia coli***	***Candida albicans***
125	0±0	10±0	0±0	0±0
250	10.1±0.5	12±1	11.2±0.2	15±1.1
500	13.8±0.14	16±1.2	13.4±0.18	20.1±0.56
1000	18.2±0.42	21.5±0.23	18.2±0.42	26.6±0.4
Negative control (distilled water)	0±0	0±0	0±0	0±0
LSD value of least significant difference	0.814			

Values represent the diameter of growth inhibition (average ± standard deviation) for five isolates of each bacterial type

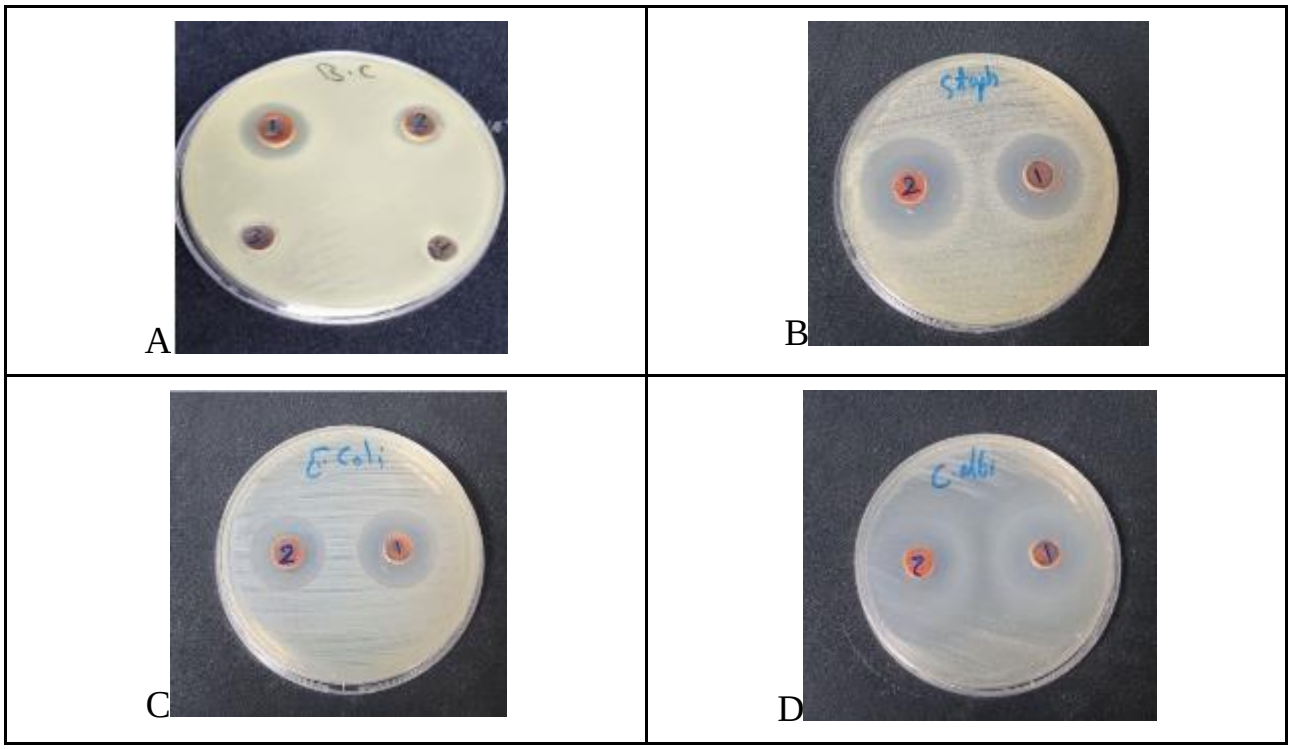

Figure 2 Sensitivity of the identified micoorgansim species to a number of antibiotics. A:*Bacilli*, B:*Staph. spp*, C:*E. coli* and D:*C. albicans*.

4. Discussion

The Antibiotic Sensitivity test, as presented in Table 2 sheds light on the susceptibility of various identified bacterial species to a spectrum of antibiotics. For *Bacilli,* Ciprofloxacin (Cip) exhibited the most pronounced inhibitory effect, with an average diameter of 28±1.02, while Amoxicillin (AX), Cefatoxime (CTX), Doxycycline (DO), and Carbenicillin (PY) demonstrated no inhibitory effect (0±0). This aligns with the findings of previous studies such as [14], who also emphasized the efficacy of Ciprofloxacin against *Bacilli* strains.

Staphylococcus displayed sensitivity to Ciprofloxacin (18±0.64), Amoxicillin (13±0.22), Cefatoxime (12±0.4), Doxycycline (12±0.4), Nitrofuranthone (THAT) (11±1), and Neomycin (17.2±1.1). These results provide valuable insights for clinicians in choosing appropriate antibiotics for treating *Staphylococcus* infections.

Escherichia coli exhibited sensitivity exclusively to Ciprofloxacin (28±1.02) and Doxycycline (19±1.4). These findings are consistent with the established susceptibility of *Escherichia coli* to fluoroquinolones like Ciprofloxacin and tetracyclines such as Doxycycline [15]. Understanding the antibiotic susceptibility of *Escherichia coli* is crucial in guiding effective therapeutic interventions.

In contrast, *Candida albicans* demonstrated resistance to all antibiotics tested. This aligns with the known resilience of *Candida albicans* to many commonly used antibiotics, as reported by [16]. The resistance pattern observed in this study underscores the challenges associated with treating *Candida albicans* infections and emphasizes the need for alternative therapeutic strategies.

The LSD value of the least significant difference was calculated as 0.911, representing the diameter of growth inhibition (average ± standard deviation) for five isolates of each bacterial type. These values highlight significant variations in antibiotic sensitivity across different bacterial species. In summary, the Antibiotic Sensitivity test provides crucial information on the efficacy of different antibiotics against identified bacterial species.

The investigation into the antimicrobial activity of SeNPs, as detailed in Table 3 and Figure 2, provides valuable insights into the inhibitory effects of nano selenium on various bacterial species.

Concentration of SeNPs: The inhibitory effects were evaluated at concentrations of 125, 250, 500, and 1000 µg/ml. 125 µg/ml: Negligible inhibition observed across all bacterial types. 250 µg/ml: Noticeable growth inhibition observed, with values ranging from 10.1±0.5 to 15±1.1, indicating a concentration-dependent response. 500 µg/ml: Increased inhibitory effects, with values of 13.8±0.14 to 20.1±0.56. 1000 µg/ml: The highest concentration exhibited the most significant inhibitory effects, with values ranging from 18.2±0.42 to 26.6±0.4. The negative control, represented by distilled water, showed no inhibitory effect, confirming that the observed effects were specifically attributed to the presence of nano selenium.

The LSD value of least significant difference (0.814) underscores the statistical significance of the observed inhibitory effects at different concentrations, further supporting the concentration-dependent response.

[17] explores the potential of SeNPs as food preservatives to reduce food spoilage. The synthesized SeNPs exhibited inhibition on the growth of Listeria Monocytogens and *Staphylococcus epidermidis* starting at 0.5 µg/mL. The inhibitory effects were concentration-dependent, aligning with the concentration-dependent response observed in the current assessment.

[18] using green-synthesized SeNPs *Vaccinium arctostaphylos* L. fruit extract was evaluated for their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Corynebacterium diphtheriae*. The study demonstrated the effectiveness of SeNPs against these bacteria, with a significant antibacterial effect observed at low MIC values. The concentration-dependent antibacterial activity aligns with the trends observed in the current assessment.

The assessment of antimicrobial activity in SeNPs, coupled with comparisons to previous studies, highlights their potential as effective agents against a range of bacterial species. The concentration-dependent response further emphasizes the versatility of SeNPs, positioning them as promising candidates for various applications, including food preservation and antibacterial agents.

5. CONCLUSION

Biological Activity (Antimicrobial): SeNPs display concentration-dependent antimicrobial activity against *Bacilli, Staphylococcus, Escherichia coli,* and *Candida albicans*. Higher SeNP concentrations lead to increased inhibitory effects on bacterial growth.

6. ETHICAL APPROVAL

Ethical approval was taken from all patients and immunocompromised patients under study, and the study was conducted in accordance with the standards recommended by (the Department of Life Sciences / College of Education / Al-Qadisiyah University and the Diwaniyah Health Department affiliated with the Iraqi Ministry of Health) in dealing with biological materials and microscopic organisms, both pathogenic and opportunistic.

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