SAR Journal of Pathology and Microbiology

Abbreviated Key Title: *SAR J Pathol Microbiol*
Home page: https://sarpublication.com/journal/sarjpm/home
DOI: https://doi.org/10.36346/sarjpm.2025.v06i04.006

ISSN 2707-7756 (P)
ISSN 2709-6890 (O)

Original Research Article

The Biological Activity of Chemogenic Selenium Nanoparticles on Salmonella enterica Isolated from Clinical Cases

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Article History: | Received: 05.06.2025 | Accepted: 11.08.2025 | Published: 13.08.2025 |

Abstract: The synthesis of chemogenic selenium nanoparticles (SeNPs) was carried out by reducing sodium selenite and ascorbic acid and properly characterized the nanoparticles to ascertain its physicochemical characteristics. X-ray diffraction (XRD) results indicated that the SeNPs were highly crystalline and hexagonal with low crystallite size, which showed its possible application in catalyst, drug delivery, and bioimaging. The scanning electron microscopy (SEM) revealed clear spherical nanoparticle SeNPs with an average size of 19.91 nm that makes them surface reactive and suitable as biomedical materials. Energy dispersive X-ray (EDX) technique was used to verify that the high purity of the SeNPs (selenium as the major element) contained oxygen, carbon, sodium, chlorine, and gold as impurities. The SeNPs tested showed a prominent dose related scavenging effect with an antioxidant activity developed in presence of DPPH in the concentration with a maximum scavenging (38.32%) at µg/mL. Investigations on hemolysis revealed that SeNPs also do not lysis red blood cells and thus has a good biocompatibility. SeNPs had MIC values on Salmonella enterica of 16 µg/mL, which indicates their antimicrobial activity. Moreover, SeNPs showed significant antibiofilm potential with Salmonella enterica and in particular in its association with tetracycline where a high degree of synergism was achieved leading to more than 80% of inhibition. The results of this study's findings indicate the promising future of SeNPs as antimicrobial and anti-oxidant application, which could possibly be used to overcome bacterial resistance as well as to act as an enhancer to the effects of conventional antibiotics.

Keywords: SeNPs, Chemogenic, Antioxidant, Antibiofilm, Hemolysis.

INTRODUCTION

In recent years, chemogenic selenium nanoparticles (SeNPs) have received much attention as these nanoparticles possess potential antimicrobial functions related mainly to their unique physicochemical properties that include small size, high surface area, and a high amount of antimicrobial activity [1]. These nanoparticles have proven to be effective against numerous pathogenic bacteria (both Gram-positive and Gram-negative bacteria) and are gaining traction regarding the possibility that they can be used in both medical and environmental practices [2]. The increased attention paid to SeNPs can be attributed not only to the direct antibacterial properties but also to the capacity to increase the antibacterial effects of traditional antibiotics especially when it comes to resisting antibiotics resistance mechanisms [3].

Salmonella enterica is a Gram-negative bacterium that is one of the most damaging bacterial pathogens in the worldwide because of its extensive association with various gastrointestinal infections (Developing). It causes morbidity and mortality and especially in the developing world since it grows into biofilm, making it cause chronic infection, and it survives when using conventional antibiotics [4]. Biofilm formation of Salmonella enterica also makes treatment more difficult since biofilms create a protective layer that increases bacterial resistance and minimizes antimicrobial uptake [5]. Thus, to improve treatment and cope with antibiotic resistance, it is of utmost importance to develop new agents that will destroy the ability to develop biofilms and prevent the growth of Salmonella, such as SeNPs.

Citation: Zahraa Alaa Hatem Al-Khazraji, Mohanad Jawad Kadhim, Mais Emad Ahmed (2025). The Biological Activity of Chemogenic Selenium Nanoparticles on Salmonella enterica Isolated from Clinical Cases. *SAR J Pathol Microbiol*, 6(4), 179-185.

In this study, we sought to examine how chemogenic SeNPs can be used in the fight against Salmonella enterica due to their antimicrobial and antibiofilm potential. In particular, the research was aimed at establishing the minimum inhibitory concentration (MIC) of SeNPs and estimating their potential to prevent the growth and biofilm formation of Salmonella. Findings of the present study add to this expanding knowledge base of the possible application of SeNPs as a competent substitute or complementary therapy in the fight against Salmonella infections, especially in relation to the resistance mechanisms through biofilms. This study seeks to offer guidelines on how SeNPs interact with Salmonella to design new antimicrobial interventions, which, along with the status quo, can help curb the menace Salmonella-related conditions pose to the world.

MATERIALS AND METHODS

Bacterial Isolation and Identification

Salmonella enterica is specimen/pathogen (Isolate) that was retrieved at clinical sources, that is, the patients who experienced diarrhea. Such isolates were first identified by a selective culture medium and were then confirmed using Vitek2. The specific primers used in verifying the diagnosis extended to the intergenic region in the 2 rRNA operons (rrnB and rrnH) that would divide the gene encoding the subunits of the ribosome (rRNA gene) in the Salmonella genomes. In this study, 14 isolates were acquired.

Chemogenic Synthesis of Selenium Nanoparticles

Chemogenic selenium nanoparticles (SeNPs) have a reduction chemical reaction with sodium selenite and ascorbic acid. Five milliliters of sodium selenite were first dissolved in deionized water giving a 50 mM solution and stabilizing agents added to deactivate the aggregation of nanoparticles and stirred magnetically for five min. The sodium selenite solution is subsequently agitated at constant agitation by the addition of 1% solution of ascorbic acid that is done gradually and at room temperature until the solution turns into a deep purple color indicating the formation of SeNPs. Under the same reaction condition, the reaction mixture is given 30 minutes as the time requirement and the formation of the nanoparticles is characterized by a color change in the mixture to reddish-brown. The nanoparticles are purified post reaction using centrifugation and the pellet is re-suspended with deionized water. By carrying out X-ray diffraction (XRD) and scanning electron microscopy (SEM), and Energy Dispersive X-ray Spectroscopy (EDX), the SeNPs were characterized to ensure that size, morphology and structural properties of SeNPs have been observed. Lastly, the SeNPs were kept in deionized water at 4 o C or lyophilized to store long term where they will remain stable to be used in other future applications [6].

Biological Activity of Chemogenic Selenium Nanoparticles

The offline DPPH assay was used to determine the antioxidant activity of the Selenium nanoparticles in line with [7], whereas the hemolysis tests were done with the blood of a healthy donor following [8]. Minimum Inhibitory Concentration (MIC) and antibiofilm activity were determined according to [9], and [10], respectively.

Statistical Analysis

One-way analysis of variance (ANOVA) was conducted to analyze data and assess the importance of difference between treatment groups. In the worst case scenario that significant effects were discovered, post hoc comparison using the Tukey method was executed to determine specific group differences using the Honest Significant difference (HSD) test. $p < 0.05$ was regarded as the level of significance of all statistical tests. All the experimental groups were represented in terms of means and standard deviation (SD). Graphical representations have been made through appropriate software and statistical significance has been represented by various letters in the bars, which indicate different groups which were not same with each other.

RESULTS AND DISCUSSION

Chemogenic Synthesis and Characterization of Selenium Nanoparticles

The XRD pattern of the chemogenic selenium nanoparticles (SeNPs) illustrated that it was slightly crystalline in nature with the predominant phase hexagonal mainly present in the SeNPs synthesized by other methods (Figure 1). The sharp XRD diffraction peaks exhibit high crystallinity and nano crystallite size, character typical of well formed nanoparticles. These observations were supported by similar studies conducted in the recent year as it has been confirmed that the chemogenic synthesis process leads to the formation of high-quality SeNPs possessing a desirable property to be applied in the field of catalysis, drug delivery, and bioimaging [11]. The XRD pattern observed confirms the phase stability and purity of the material as well as the low value of the crystallite size implying the possibility of size dependent functionalities [12].

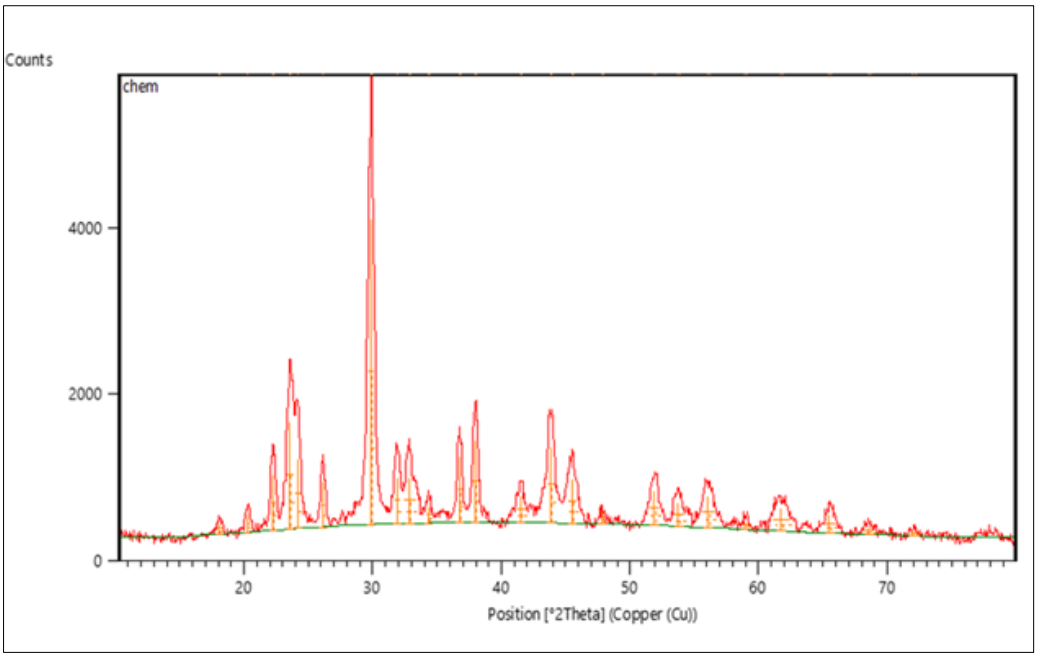

Figure 1: The X-ray diffraction (XRD) of Chemogenic SeNPs

The SEM image of the chemogenic selenium nanoparticles (SeNPs) reveals nanoparticles with an average size of approximately 19.91 nm (Figure 2). The particles appear well-defined and uniform, suggesting successful synthesis with minimal aggregation. This small size enhances the nanoparticles' surface reactivity, which is beneficial for various applications, including biomedical, catalytic, and environmental fields [13]. The size and morphology observed here align with typical characteristics of SeNPs synthesized through chemical reduction methods, making them suitable for applications that require high surface area and reactivity [14].

The EDX spectrum of chemogenic selenium nanoparticles (SeNPs) showed that selenium is the principal element, which certifies a high-purity SeNPs formation (Figure 3). Oxygen, carbon, sodium, chlorine and gold can also be found in the spectrum (Figure 2). The presence of oxygen and carbon are most probably the byproducts of surface oxidation and capping organic agents respectively, and the chlorine and sodium may be by products of the synthesis process. The analysis utilizes conductive coating, which is made of gold. The general element content corresponds to anticipated features of selenium nanoparticles, sheds light on their pureness and character, that are critical to potential use in purposes, such as catalysis, drug distribution, and biomedicines [13].

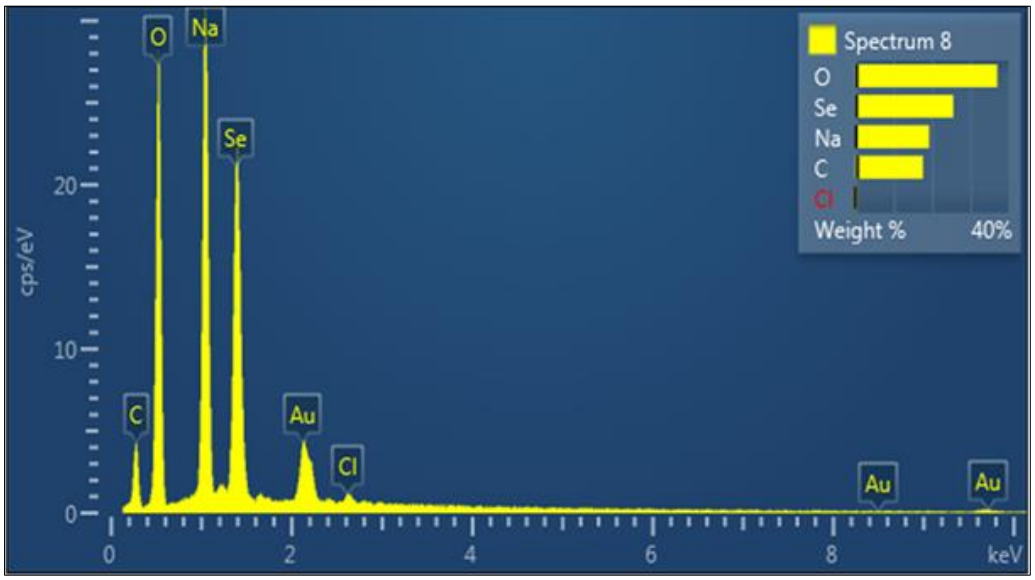

Figure 2: The Scanning Electron Microscopy of Chemogenic SeNPs

Figure 3: The Energy dispersive X-ray (EDX) spectrometry of Chemogenic SeNPs

Antioxidant activity of Chemogenic SeNPs

Chemogenic selenium nanoparticles (SeNPs) contain high antioxidant activity as determined through the DPPH assay which increases with an increase in the doses. The percentage of scavenging at 4 µg/mL is 10.39 % and 38.32 at 64 µg/mL, rendering the notion that SeNPs have considerable antioxidant capacity in high concentration Table 1. These findings revealed a direct relationship between concentration and antioxidant ability by giving the greatest result of scavenging power at 64 µg/mL. These results were in agreement with available literature concerning selenium nanoparticle and showed the potential that the nanoparticles hold as good antioxidants in numerous biomedical components [15].

Table 1: The antioxidant activity of Chemogenic Selenium nanoparticles using DPPH test

Concentration of SeNPs	Absorbency	Scavenging %
4	1.372	10.39
8	1.372	11.48
16	1.327	14.93
32	1.334	13.94
64	0.956	38.32

Hemolysis Activity of Chemogenic SeNPs

The hemolysis activity of chemogenic selenium nanoparticles (SeNPs) was evaluated at concentrations ranging from 4 to 64 µg/mL, and the results showed no significant hemolysis, as indicated by the hemolysis percentages of 0% and absorbency values between 0.5960 and 0.9291 Table 2. These findings suggested that SeNPs do not induce red blood cell lysis at these concentrations, indicating their good biocompatibility. The positive and negative controls confirmed the validity of the assay, with the positive control causing complete hemolysis (100%) and the negative control showing no hemolysis. These results highlight the low cytotoxicity of SeNPs, making them suitable for biomedical applications that require compatibility with human blood cells [1].

Table 2: The hemolysis activity of Chemogenic Selenium nanoparticles against human RBC

Concentration of SeNPs	Absorbency	Hemolysis %
4	0.9291	0
8	0.7736	0
16	0.6998	0
32	0.6990	0
64	0.5960	0
Positive	2.5446	100
Negative	0.566	0

The MIC of Chemogenic SeNPs against Salmonella Enterica

The MIC of chemogenic selenium nanoparticles (SeNPs) against Salmonella enterica indicated that SeNPs possess considerable anti-microbial effects, since the lowest active concentration was recorded in the case of 16 µg/mL. No growth was evident at this concentration hence there was maximum inhibition of growth of Salmonella. There was not a further increase in inhibition observed at higher concentrations (32 µg/mL and 64 µg/mL) and therefore a maximum of 16 µg/mL is all that is needed to achieve maximum antimicrobial effect Table 3. The results indicated the usefulness of SeNPs as antibacterial against Salmonella enterica, which could be used in the treatment of bacterial infections. The noted MIC (16 µg/mL) was comparable to that found in other publications regarding the antimicrobial action of SeNPs, and it was revealed that bacterial cultivations were inhibited established, including S. enterica, in a dose-dependent pattern [16]. The findings indicated that SeNPs can be easily used to suppress bacterial growth, with comparatively low concentrations, which supports the capability of antimicrobial agents in the treatment of infections triggered by Salmonella enterica [17].

Table 3: The MIC of Chemogenic SeNPs against Salmonella enterica

SeNPs Concentration (µg/mL)	OD (600 nm)	Interpretation	MIC Determination
0 (Negative Control)	1.00	Full microbial growth	No inhibition, base OD value
4	0.90	Moderate growth, inhibition begins	Growth slower moderate inhibition
8	0.80	Reduced growth, more inhibition	Less growth, stronger inhibition than previous
16	0.60	No increase in OD compared to previous value	No growth increase, MIC observed here
32	0.40	No increase in OD compared to 16 µg/mL	No further growth, concentration consistent with MIC
64	0.40	No increase in OD compared to 32 µg/mL	No further growth, concentration consistent with MIC

Antibiofilm Activity of Chemogenic SeNPs against Salmonella Enterica

The results in the figure 4 proved that chemogenic selenium nanoparticles (SeNPs) have considerable antibiofilm activity against Salmonella enterica, and its biofilm was inhibited moderately at MIC and sub-MIC. In combination with tetracycline (TET), at MIC or lower concentrations, the SeNPs added antibiofilm activity attains over 80% in MIC levels with strong synergy effect. By contrast, tetracycline alone was weakly biofilm inhibitory. As evidenced by statistical analysis, the combination of SeNPs and tetracycline proved to be quite effective, and thus the potential of SeNPs as supplements that can be added to conventional antibiotics to increase their effectiveness in the treatment of biofilm-related infections caused by Salmonella enterica clearly exists.

The synergic antibiofilm effect in this study conformed to prior studies where it was reported that nanoparticles, as SeNPs, are capable of augmenting the effectiveness of standard antibiotics. Such combination therapy is proven to enhance the molecule penetration of antibiotics through biofilms and decrease the microbial resistance [18]. Nanoparticles of selenium have also been reported to interfere with biofilm formation and improve the capacity of antibiotics [19]. Such synergistic action is explained by the fact that SeNPs can interfere with the walls of bacterial cells and help antibiotics enter the biofilm [18].

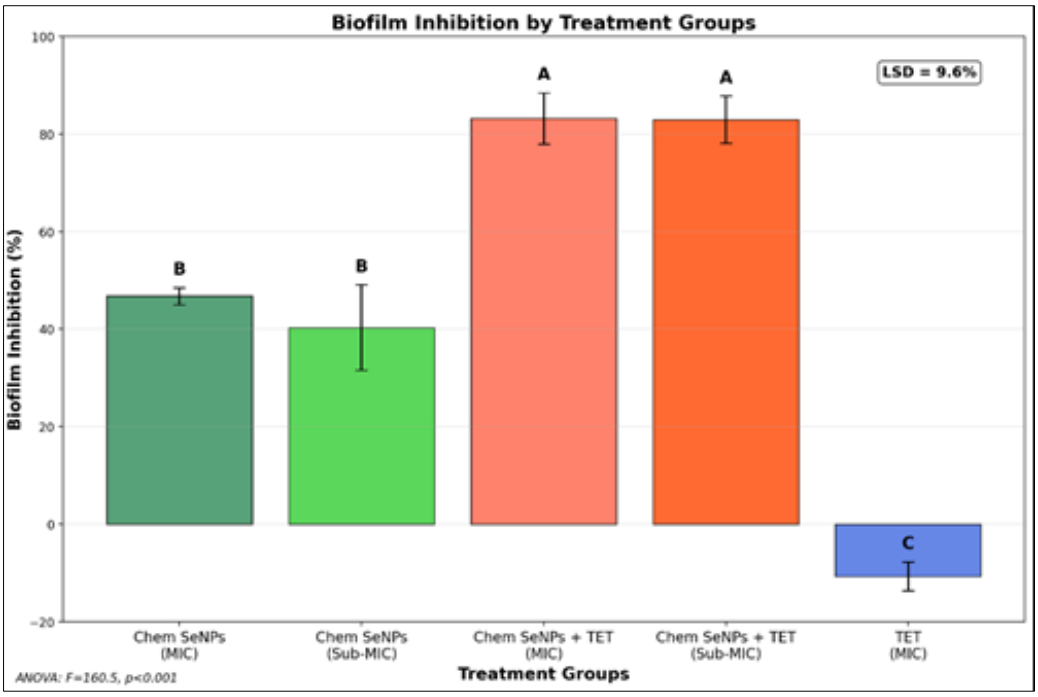

Figure 4: The Biofilm inhibition percentages of Chemogenic SeNPs against *Salmonella enterica* in comparison with tetracycline antibiotic. Bars represent means ± SD (n = 3). Similar and Different letters represent groups that are not significantly and significantly different, respectively by ANOVA LSD

CONCLUSION

High crystallinity and hexagonal phase were observed in the selenium nanoparticles (SeNPs) using the chemogenic method of preparation, but these particles are indicated to be useful in catalysis techniques, drug delivery, and bioimaging due to their dimensions of 19.91 nm. The SeNPs had a high dose-dependent antioxidant activity and had no hemolytic activity, which implies their biocompatibility. It was established that the minimum inhibitory concentration against Salmonella enterica was 16g/mL and it has significant antimicrobial potential. Also, SeNPs showed stronger anti biofilm properties especially in conjunction with tetracycline suggesting they can complement the action of traditional antibiotics. These results indicate that SeNPs have a significant potential to be applied therapeutically and industrially, particularly to fight infections and biofilm resistance-linked resistance.

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