RESEARCH PAPER

The Biological Activity of Biogenic Selenium Nanoparticles on *Salmonella Enterica* Isolated from Clinical Cases

Zahraa Alaa Hatem Al-khazraji [1], Mais Emad Ahmed [2], Mohanad Jawad Kadhim [1]*

[1] *Department of Applied Biotechnology, College of Biotechnology, Al-Qasim Green University, Babylon, Iraq*
[2] *Department of Biology, College of Science, University of Baghdad, Jadriya, Baghdad, Iraq*

ARTICLE INFO

Article History:
Received 24 May 2025
Accepted 26 September 2025
Published 01 October 2025

Keywords:
Antibiofilm
Salmonella enterica
SeNPs
XRD

ABSTRACT

This research examined the identification of biogenic selenium nanoparticle (SeNPs) synthesized with lemon peel extract, the antimicrobial and antimicrobial biofilm inhibition test conducted on Salmonella enterica isolates, the prepared surface confirmed by XRD, EDX and FESEM techniques. The selective media committed in identification of the Salmonella isolates were SS and XLD whereby they exemplified normal colony morphologies characteristics of living organisms producing sulfur (hydrogen sulfide is produced by Salmonella species). Biochemical identification was also authenticated using Vitek2 Automated analysis and the probabilities given are similar to the Salmonella species identification that is 94-99% and were confirmed using molecular tools that is gel electrophoresis and NCBI BLAST analysis where high percentages of identity were shown 93.41% to 99.75% to Salmonella enterica subsp. enterica. The results of antibiotic susceptibility testing revealed high uncertainty in resistance pattern as far as some of the isolates were multiple-drug resistant (MDR), which indicates the increasing problem of antibiotic resistance related to Salmonella. Antimicrobial properties of biogenic SeNPs were also determined and it was identified that antimicrobial action of the nanomaterials was dose-dependent with minimum inhibitory concentration (MIC) of 32 µg/mL. Furthermore, the SeNPs had a high inhibitory effect on the biofilm formation that was also synergistic once combined with tetracycline. These data indicate that biogenic SeNPs are promising antimicrobial agents and add-ons to conventional antibiotics against Salmonella infection and biofilm-related resistance.

How to cite this article
Al-khazraji Z., Ahmed M., Kadhim M. The Biological Activity of Biogenic Selenium Nanoparticles on Salmonella Enterica Isolated from Clinical Cases. J Nanostruct, 2025; 15(4):2492-2503. DOI: 10.22052/JNS.2025.04.085

INTRODUCTION

Nanotechnology is a science that involves nanoparticles or an object that has less than nanometer (NPs). Nanomaterials are small solid particle sizes of one to one hundred nanometers. Because of their improved one-of-a-kind physicochemical properties, such as their minuscule size, significant surface area in proportion to volume, and higher reactivity, nanomaterials have the potential of numerous uses to human welfare [1].

Selenium nanoparticles (SeNPs) have also

* *Corresponding Author Email: dr.mohanad@biotech.uoqasim.edu.iq*

attracted a lot of attention towards the sphere of nanotechnology because of their properties, and diverse applications [2]. The green synthesis of SeNPs has certain benefits especially, in the situation when the rising demands of the environmentally-friendly, incomparable non-toxic, and affordable approaches are considered where the method fits into the general picture of the environmentally friendly nanomaterials [3]. The antimicrobial, antiviral, anticancer, antioxidant, and anti-inflammatory effect, among others, are some of the excellent biomedical applications of SeNPs as highlighted in recent advancements in research [4]. These nanoparticles have high promise of therapeutic value and it is important to learn the mechanism by which these are produced. These kinds of insights can become the basis of new treatment techniques that have a broad scope of application in treatment methods [5].

Green nanotechnology has been described as a transformative principle whose goal is to create sustainable technologies that are designed to reduce the health and environmental risks posed to such products manufactured through nanotechnology and such products being applied. This proposal insists on the necessity to substitute traditional products with nanomaterials that are easy to the environment in all their life cycle; synthesis through to waste disposal [6]. Parallel to this, a look to engineering and chemistry is evident, where the principle of green chemistry has found its way as a guideline in chemical research and product development, to design chemicals and processes with a minimum potential impact on health and to the environment, i.e., they make up a minimum ecological foot print [7]. Where environmental chemistry seeks to understand the chemistry of the natural world and pollutants, green chemistry extends this to include other chemical, organic, inorganic, biochemical, analytical and physical chemistries in order to work at the root of the pollution problem rather than the mitigation of the consequences [8].

Salmonella enterica is a potent bacterial pathogen that has been causing numerous infectious diseases in both people and animals. It harbors more than 2,500 serovars, and some of them such as Salmonella enterica serovars Typhi and Typhimurium are key causes of foodborne infections in the world [9]. These infections occur as either gastroenteritis or an enteric fever which has diarrhea, abdominal cramps, fever and vomiting. Although the majority of the breakdowns are self-limiting, some serovars can easily cause serious complications, such as bacteremia and septicemia, especially in vulnerable groups, like the aged people, immunocompromised patients, and the very young children [10]. The major modes of transmission include consumption of infected foodstuff, especially undercooked chicken, eggs, and direct contact with ill animals [11]. S. enterica resistance to these environments and its abilities to develop resistance to antimicrobials also make the task of control more difficult. Hence, S. enterica has been a priority area of public health research to decrease the burden of foodborne diseases by Mattassi et al. practicing food safety and antimicrobial stewardship [12].

The aim of the current research was to evaluate the antibacterial activity of the biogenic selenium nanoparticles (SeNPs) against clinical strains of Salmonella enterica. With this sort of investigation, we aimed to understand the possibilities of using SeNPs as a powerful antimicrobial agent along with gaining a better understanding of the effect of SeNPs on this pathogenic bacterium.

MATERIALS AND METHODS

Specimens Collection and Bacterial Identification

The total number of Salmonella isolates obtained was 14 of which 10 were isolates of the College of Science, the University of Baghdad and the other 4 were of the College of Science the University of Al-Qadisiyah during the period between October and December 2024. Each of the isolates was cultivated in stool samples of patients who attended to them with symptoms of diarrhea. Identification of the strains of Salmonella was then done via two selective agar plates namely SS (Salmonella-Shigella) and XLD (Xylose Lysine Deoxycholate) [13] followed by confirmation of the results by molecular methods. Specifically, the primer pair Sal-F (5′-CGATGCGTTGAGCTAACCGG-3′) and Sal-R (5′-CAGAAGCGATAACCACGTCGTC-3′) was employed to amplify a 400–900 bp fragment encompassing the intergenic region in 2 rRNA operons (rrnB and rrnH) that separates DNA-encoding ribosomal subunits (rRNA gene) in Salmonella genomes. Polymerase chain reaction (PCR) amplification was performed using the GoTaq® Green Master Mix (Promega), following standard preparation protocols. The thermocycling profile consisted of an initial denaturation step at

95°C for 7 minutes, followed by 35 amplification cycles (denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute), and a final elongation step at 72°C for 7 minutes. The resulting PCR products were subjected to Sanger sequencing, and sequence data were subsequently analyzed using the BLAST tool provided by the National Center for Biotechnology Information (NCBI) [14].

Antibiotic Susceptibility and Biofilm formation Test of Salmonella enterica

The antibiotic susceptibility test was performed on all *Salmonella enterica* isolates against 9 antibiotics and the antibiotic resistance patterns were determined according to [15]. Quantitative biofilm formation tests were achieved according to Stepanovic [16].

Biogenic synthesis of selenium nanoparticles using Lemon peels extract

This procedure performed according to [17] as follow: After washing and shredding, the GLP was dried at 60°C for 12 hours until a constant weight was achieved. Subsequently, 50g of the dried GLP was combined with 1L of distilled water and heated to various temperature levels (60–80°C) for different durations (1–3 hours) under continuous stirring. The resulting lemon peel extract was then filtered and stored at 4°C for subsequent experiments. Selenious acid (H_2SeO_3, >99.9%), gallic acid ($C_6H_2(OH)_3COOH$, >97.5%), and the Folin–Ciocalteu phenol reagent were sourced from Merck. The polyphenol concentration in the GLP extract was quantified using the colorimetric method with the Folin–Ciocalteu reagent, employing gallic acid as the calibration standard. The presence of polyphenols was determined using a UV-Vis spectrophotometer (UV-1800, Shimadzu) at a wavelength of 760 nm.

To synthesize SeNPs, a specific volume (VExt, mL) of the GLP extract was combined with a corresponding volume (VSe, mL) of H_2SeO_3 solution at a concentration (CSe, mM) under stirring at 300 rpm for 30 minutes at room temperature to obtain a homogeneous solution. After stirring, the solution was transferred to a Teflon autoclave for hydrothermal treatment at a designated temperature (T°C) for a specified time (t). The effects of the volume ratio of extract to selenious acid solution (VExt/VSe), the concentration of selenious acid (CSe), the duration of the reaction (t), and the synthesis temperature (T) on the formation of SeNPs were systematically investigated to identify the optimal conditions for nanoparticle synthesis.

Characterization of Biogenic SeNPs

To fully examine the structural and morphological characteristics of the selenium nanoparticles a broad set of characterization was done. Such techniques included scanning electron microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX) [18].

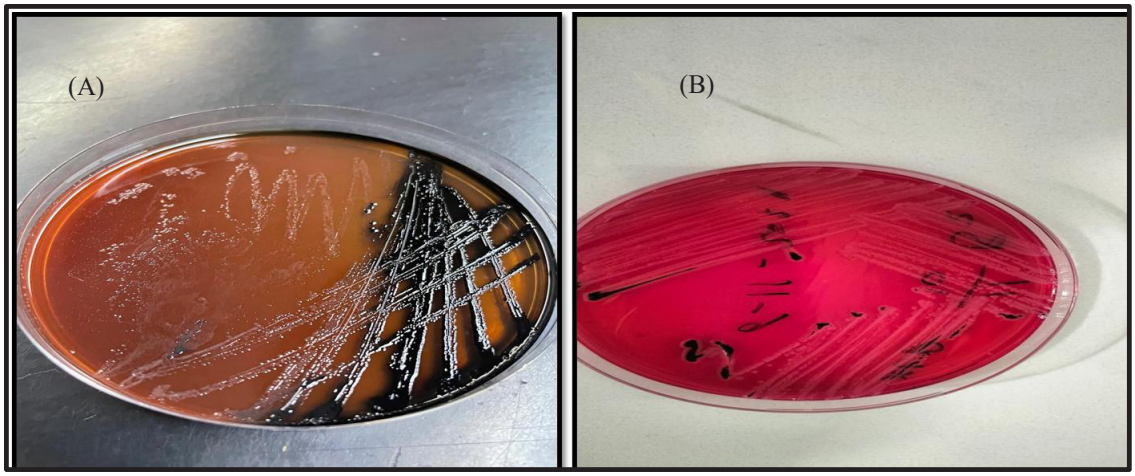

Fig. 1. Characteristic features of Salmonella spp. A: on Salmonella-Shigella (SS) agar, B: on Xylose Lysine Deoxycholate (XLD) agar after 24 hours of incubation at 37 ^{0}C.

Biological Activity of SeNPs

Minimum Inhibitory Concentration (MIC)

The broth microdilution technique was used to determine Minimum Inhibitory Concentration (MIC) of the synthesized selenium nanoparticles (SeNPs) against Salmonella enterica strains. The process was done as serial dilutions of SeNPs in culture broth concentrations of 64, 32, 16, 8 and 4 ug/ml were prepared. The actions that were undertaken were as follows [19]:

1. A stock solution of SeNPs (64 µg/ml) was prepared, from which 100 µL was transferred into the first row of wells in a 96-well microplate.
2. To each well (1 to 10), 100 µL of Müller-Hinton broth was added, followed by a two-fold dilution of SeNPs and tetracycline, achieved by transferring 100 µL from one well to the subsequent well. In the microtiter plate, Well G served as the positive control, while well H was designated as the negative control.
3. A bacterial suspension of *Salmonella enterica* was adjusted to a turbidity of McFarland 1.5 (1.5 × 10^8 CFU/ml) using a densitometer. Ten microliters of the bacterial suspension were inoculated into each well, except for the negative control well.
4. The microtiter plate was incubated at 37°C for 24 hours, after which bacterial growth was assessed by measuring the optical density at 450 nm (OD450) using a microtiter plate reader.
5. The concentration at which no increase or change in optical density (OD) in comparison to the previous concentration was considered the Minimum Inhibitory Concentration (MIC).
6. Tetracycline antibiotic was used for comparison with biogenic SeNPs, and its MIC (6.4 µg/ml) was prepared according CLSI.

Antibiofilm Activity of Selenium Nanoparticles Against Salmonella enterica

The antibiofilm effectiveness of SeNPs, TET against an MDR *Salmonella enterica* isolate had been tested using plate tissue culture method, which described by Basumatari and his coauthors [20].

Statistical Analysis

A variety of statistical methods were used in order to analyze the data properly, thus making it robust and reliable. Mean, standard deviation,

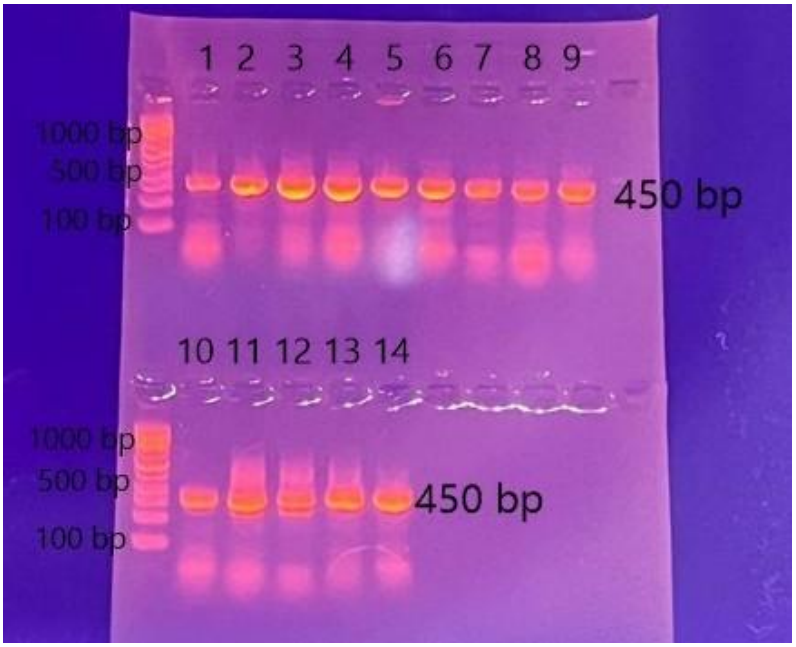

Fig. 2. The gel electrophoresis of intergenic region in 2 rRNA operons (rrnB and rrnH) with a specific location upstream of the dkgB gene of Salmonella entireca. (1-14) represent the number of isolates. 450 bp represent the amplicon size. The conditions were Agarose 1.5%; Volt. 100; Amp. 80; time 1 hour.

and range were used as descriptive statistics and one-way ANOVA as well as one-way ANOVA with Tukey HSD were used to compare mean values of multinomial populations. Kruskal-wallis test was used in case of data that were not normally distributed. Chi-square analysis was done to determine the relationships among the categorical variables which included multiple drug resistance (MDR) strains. Even where differences were not statistically significant, the biological significance of that parental effect was quantified as an effect size measure (Cohen d). The modeling of the relationship between SeNP concentration and its antimicrobial effects was done using the linear regression. A p significance of less than 0.05 was to be used, with p value showing extreme significance at less than 0.001. All these analyses were complemented by data visualization using bar charts, histograms, and scatter and made it easy to understand the data found.

RESULTS AND DISCUSSION

Identification of Salmonella enterica isolates

The primary identification results in Fig. 1 depicted growth of Salmonella spp. on two selective media: SS (Salmonella-Shigella) and XLD (Xylose Lysine Deoxycholate) agar. On SS agar, colonies of Salmonella had dark interior with colourless or slightly translucent edges which implied presence of hydrogen sulfide (H 2 S). The black centers are produced because the sodium thiosulfate is reduced, and it produced H2S that reacted with iron salts present in the medium and forms a black precipitate [21]. The presence of Salmonella was identified on XLD agar with red colonies and black centers, indicating that Xylose was fermented with production of H2S by this pathogen [22]. These results were similar to other works that also utilized these media in the selective isolation of Salmonella in other enteric bacteria. The ability of reading H2S production is a classical biochemical feature of assistance in Salmonella identification in microbiology diagnosis [21,22]. Both media were effective in the susceptibility of Salmonella in clinical and food microbiology helping in fast diagnosis of possible foodborne diseases.

An efficient amplification of 450 bp fragment in Salmonella isolates was confirmed by gel electrophoresis Fig. 2 as intergenic region of in 2 rRNA operons (rrnB and rrnH) that separates DNA-encoding ribosomal subunits (rRNA gene) in Salmonella genomes. The bands that were raised were equal to that which was expected, and is indicative of the successful PCR products. The identity of the isolates was further verified

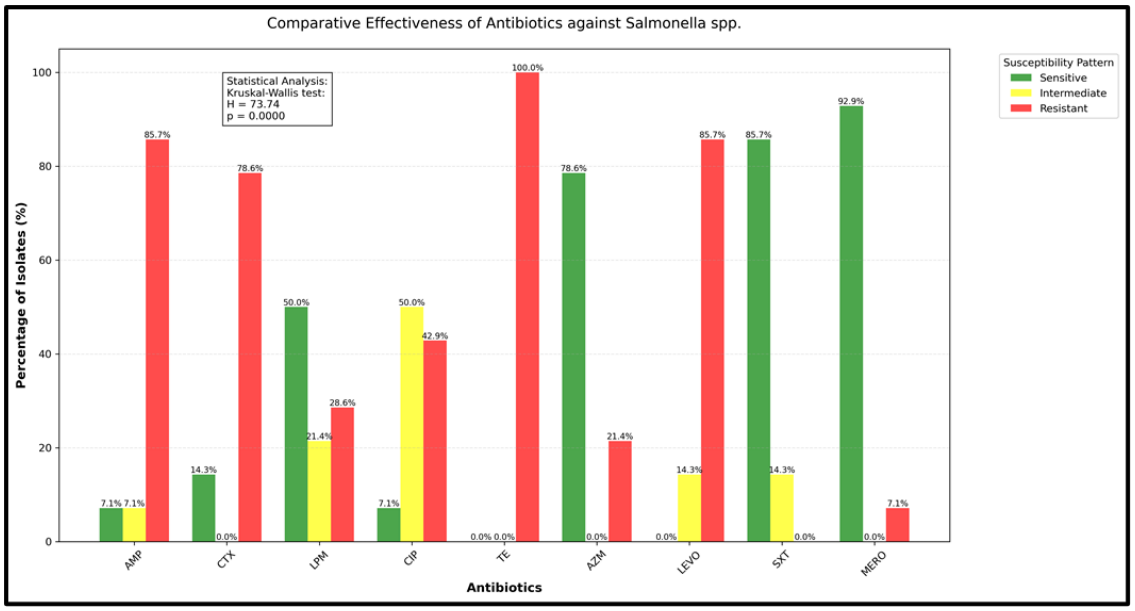

Fig. 3. Comparative effectiveness of antibiotics against Salmonella spp. AMP: Ampicillin; CTX: Cefotaxime; IPM: Imipenem; CIP: Ciprofloxacin; TE: Tetracycline; AZM: Azithromycin; LEVO: Levofloxacin; STX: Trimethoprim/ sulfamethoxazole; MERO: Meropenem

Table 1. The NCBI results for the identification of Salmonella spp. the results were interpreted with *Salmonella enterica* subsp. enterica (CP182171.1).

Local isolate Number	Standard strain	E-value	Identity percentage
1	*Salmonella enterica* subsp. enterica (CP182171.1)	0.0	93.41 %
2		2e-138	98.61 %
3		4e-139	98.95 %
4		0.0	95.11 %
5		2e-137	99.64 %
6		0.0	95.50 %
7		3e-120	96.68 %
8		3e-126	96.50 %
9		2e-138	98.28 %
10		0.0	97.37 %
11		0.0	96.10 %
12		0.0	89.88 %
13		1e-139	98.95 %
14		2e-122	96.42 %

by an NCBI BLAST analysis, and a small range in the percentage of identity was observed ranging between 93.41% and 99.75% as compared to the Salmonella enterica subsp. enterica reference strain (CP182171.1) with low E-values as an indication of strong significance of alignment Table 1. Isolate 5 achieved the finest identity (99.64%) to affirm validity of the sequencing outcome. The results were in line with the existing researches on Salmonella identification using rRNA gene sequencing as 23S rRNA genes is preserved and possess variable intergenic locations, thus representing dependable markers [14].

Antibiotic susceptibility of Salmonella enterica

This study has presented the efficacy of nine of these antibiotics against Salmonella spp and showed that they vary greatly in terms of susceptibility (Fig. 3). According to the Kruskal-Wallis test, a significant difference between these antibiotics was observed (H = 73.736, p = 0.000). Highest sensitivity rate was observed with Trimethoprim/sulfamethoxazole (SXT) and Meropenem (MERO) at 85.7 percent, whereas Tetracycline (TE) displayed 100 percent drug resistance and as such it can no longer be employed. Ciprofloxacin (CIP) and Ampicillin (AMP) were also found to be highly resistant which signify their ineffective effect. As pairwise comparisons, all antibiotics varied in sensitivity against the others but with AZM having 78.6 percent susceptibility. These indicated that the effectiveness of antibiotic interactions depended on doses. The research pointed out the significance of susceptibility testing and keeping the area resistance pattern in mind before prescribing antibiotics, as SXT, MERO, or AZM possibly is used as the first-line treatment. These results were congruent with the international data on Salmonella resistance [23], and Tetracycline resistance is consistent with the widespread resistance being recorded [24]. The efficacy of SXT and MERO makes them significant in their application against resistant strains, but Ciprofloxacin and Ampicillin resistance is a developing situation which should prompt a response in their guidelines as well [25]. The presence of variability in Azithromycin resistance indicates that personalization of therapy is paramount and constant surveillance and careful antibiotic use is required in controlling resistance to Salmonella [23].

The study also revealed that Multiple Drug Resistance (MDR) isolates had the 92.86% in the study, Non-MDR isolates had the remainder 7.14% (Fig. 4). The Chi-squared test (p = 4.50e-33, 143.53) indicated that MDR and Non-MDR isolates significantly differed. The results, predictive of global issues, concerning the prevalence of MDR underscore the need to continue surveillance and poor antibiotic stewardship to prevent prevention of the spread of resistant bacteria [26]. The rising antibiotic resistance to the first-line antibiotics necessitates intensive monitoring and sensible consumption of antibiotics to counter this emerging peril [26].

The Biofilm formation Degree of Salmonella enterica Isolates

This research studied the biofilm production of Salmonella spp. isolates which is considered

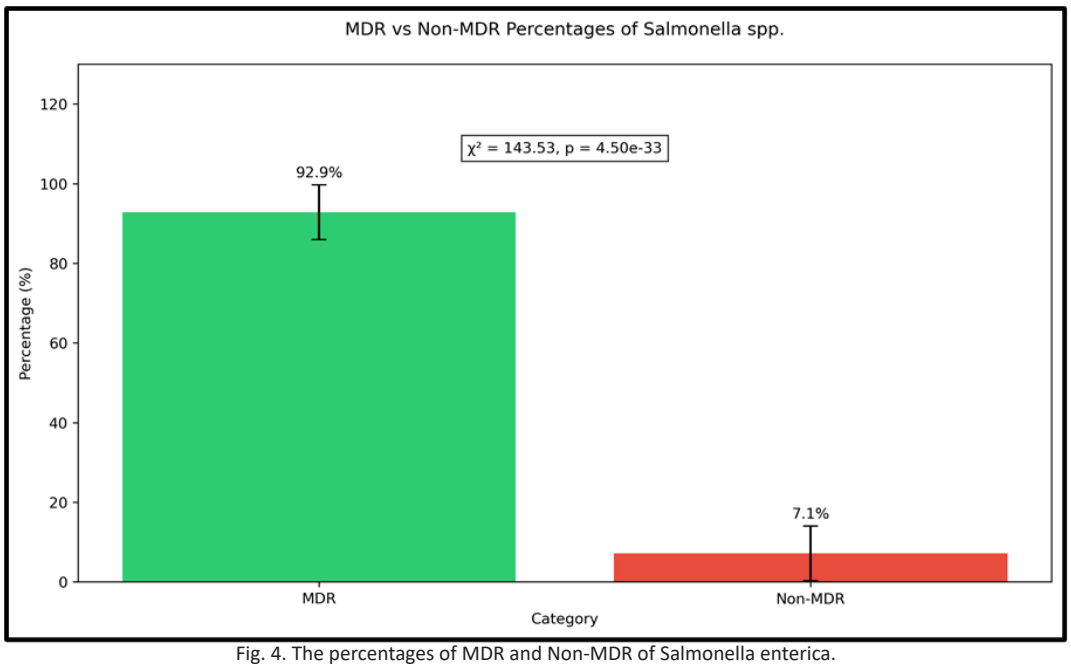

Fig. 4. The percentages of MDR and Non-MDR of Salmonella enterica.

one of the virulent factors associated with chronic diseases and resistance to antimicrobials (Fig. 5). The optical density (OD) was used to measure the biofilm formation and classified into strong, moderate, weak, and non-producers. The outcome demonstrated that 35.7 percent of the respondents were strong, 57.1 percent moderate, and 7.1 percent weak biofilm producers. The results of one-way ANOVA and Tukey HSD post-hoc indicated that the categories significantly differ ($F = 763.6555$, $p < 0.001$), which means that the biofilm formation widely varies. The strongest OD came when the strong producers were compared to the weak ones, which illustrates how biofilm-forming isolates virulent, and increase resistance to antibiotic and immune system response of the host. The results are consistent with those of other studies in which Salmonella had considerable differences in biofilm production both in Iraq and worldwide [27]. Biofilm develops in associations with chronic infections and aggravated antibiotics (ciprofloxacin and ampicillin) resistance [26]. High biofilm producers like isolates S1, S 2 and S 6 pose a greater problem in the treatment and management of salmonella infections through high rates of virulence. These results reiterate the fact that this phenomenon of biofilm formation should be addressed during infection control and antibiotic stewardship initiatives.

Biogenic Synthesis and Characterization of Selenium Nanoparticles

Figs. 6 to 8 were presented to characterize the biogenic selenium nanoparticles (SeNPs) formed with the lemon peel extract. X-ray diffraction (XRD) analysis demonstrated crystalline particles, and hence an appropriately defined structure of SeNPs (Fig. 6). The SEM pictures showed that nanoparticles are spherical shaped and had an average size of about 24.56 nm and the histograms proved that small nanoparticles dominate (Fig. 7). EDX analysis showed selenium as the most abundant element and traces of other elements such as carbon, oxygen, etc (Fig. 8). Lemon peel extract is a new method of green nanotechnology in producing SeNPs that is environment-friendly and it is economical compared to other methods. Successful synthesis of SeNPs using plant materials is also noted in previous studies as a biocompatible, simple to produce material [28]. The diameter of the SeNPs in this research (24.56 nm) is consistent with the other green synthesis methods since smaller sizes support better bioactivity [29]. These SeNPs have high purity and maintenance of structure as shown in XRD patterns [28]. All these results illustrate the lemon peel extract as a potent

and renewable reducing agent and thus the SeNPs are promising nanomaterials with potential use in nanomedicine and environmental remediation.

The Minimum Inhibitory Concentration of Selenium Nanoparticles

The dose-dependent antimicrobial action of biogenic selenium nanoparticles (SeNPs) was peaked and realized using the MIC assessment. At negative control, the OD 600 was 1.00 which is the maximum growth of the microbe. With the rise in concentration of SeNP, there was a reduction in OD: 0.90 at 4 µg/mL, 0.80 at 8 µg/mL and 0.60 at 16 µg/mL. Microbial growth was inhibited when the concentration reached 32 0g/mL and no additional decrease of OD was recorded at 64 0g/mL, which explains that MIC was 32 µg/mL. This would mean that SeNPs synthesized through lemon peel extract cannot be ignored in limiting the growth of microbes, probably because of their tiny size, large surface area and bioreactive surface chemistry. The MIC value of the sample against Salmonella enterica 32 µg/mL and was in line with the reports on biogenic SeNPs. Similar SeNPs were found to have 25-50 MIC against MDR clinical pathogens [30] and against foodborne pathogens [31]. One study reviewing the antibacterial effectiveness of SeNP mentioned that green-synthesized nanoparticles have their MIC at a low level (less than 100 100 µg/mL) especially when the capping agent is of biological origin and in the case of plant extracts [32]. Nonetheless, other works with artificially produced SeNPs were not as sensitive in S. enterica where MIC levels were beyond that of a standard range [33]. Contrary to this finding, an even measurably less potent antimicrobial effect of biogenic SeNPs was supported by lower MIC values (~25 µg/mL) against S. enterica and S. typhi when different SeNPs were synthesized using citrus peel extracts (e.g., orange or cinnamon) [34]. In this regard, the MIC of 32 u g / mL obtained in this concept therefore agreed with the increased activity of the plant extract synthesized SeNPs and underscores their potential as Salmonella enterica

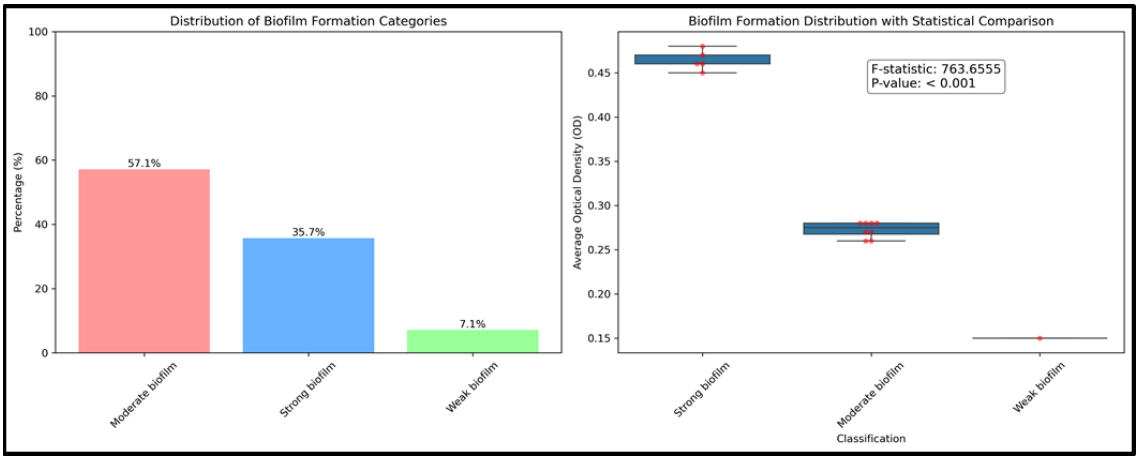

Fig. 5. Distribution of Salmonella spp. biofilm formation categories (left) and statistical comparison of optical density values across categories (right). Statistical analysis shows significant differences between groups (F = 763.6555, p < 0.001).

Table 2. The minimum inhibitory concentration (MIC) of SeNPs against Salmonella enterica by suing tissue culture plate method.

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	Significant growth, but slight inhibition	Growth observed, slight inhibition
8	0.80	Moderate growth, inhibition begins	Growth slower than previous, moderate inhibition
16	0.60	Reduced growth, more inhibition	Less growth, stronger inhibition than previous
32	0.40	No increase in OD compared to previous value	No growth increase, MIC observed here
64	0.40	No increase in OD compared to 32 µg/mL	No further growth, concentration consistent with MIC

antimicrobial agents.

Antibiofilm Activity of Biogenic SeNPs against Salmonella enterica

Apart, the current study showed biofilm inhibition of the biogenic selenium nanoparticles (SeNPs) on Salmonella enterica as results presented in Fig. 9 showed biofilm inhibition across the treatment groups. The SeNPs at the same minimum inhibitory concentration (MIC) of

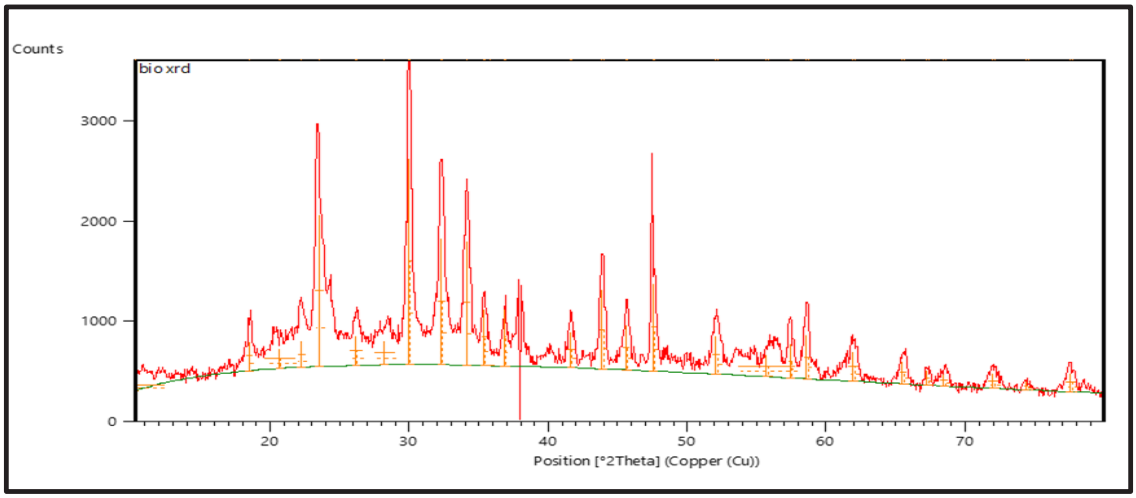

Fig. 6. The X-ray Diffraction (XRD) Analysis of Crystalline Structure of Biogenic Selenium Nanoparticles.

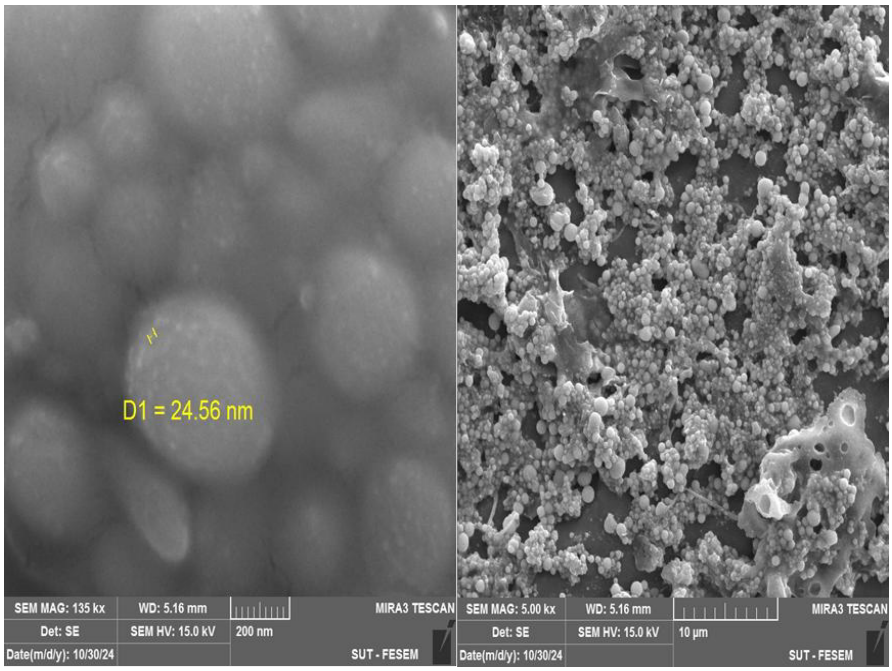

Fig. 7. Scanning Electron Microscopy (SEM) Imaging of Biogenic Selenium Nanoparticles: Surface Morphology and Particle Size.

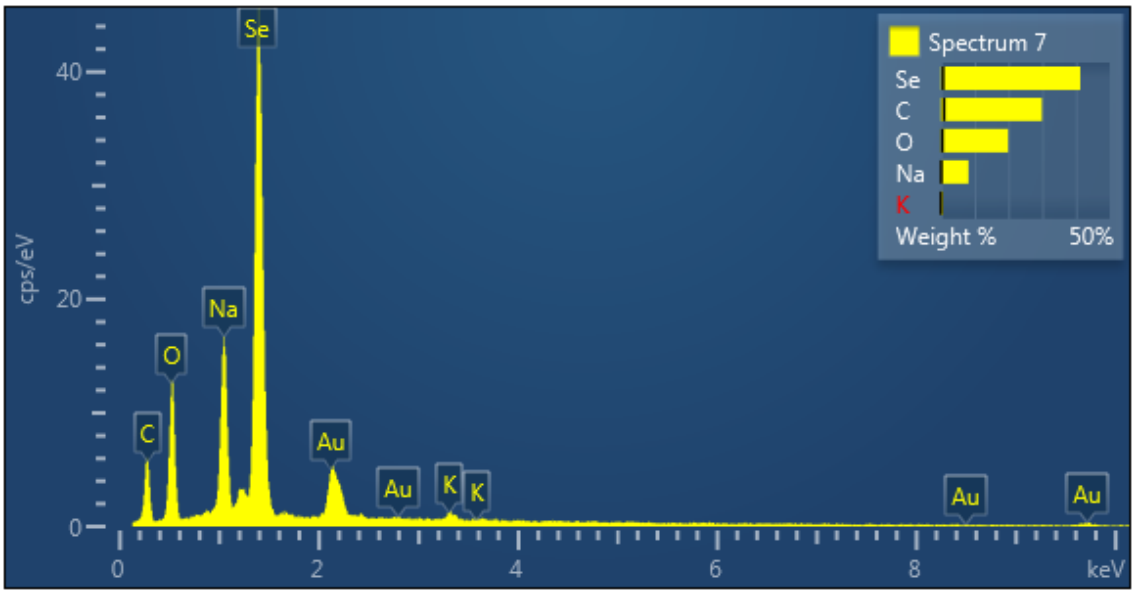

Fig. 8. Energy Dispersive X-ray Spectroscopy (EDX) for Elemental Composition Analysis of Selenium Nanoparticles.

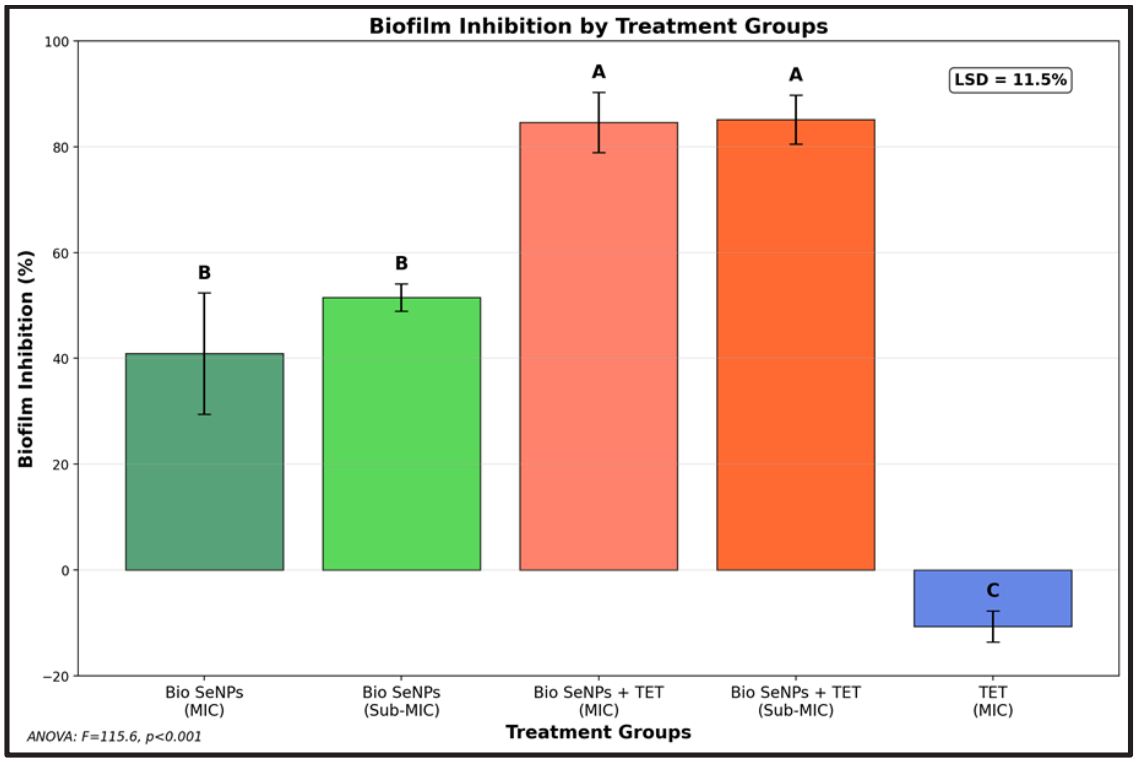

Fig. 9. The Biofilm inhibition percentages of SeNPs against *Salmonella enterica* in comparison with tetracycline antibiotic. Bars represent means ± SD (n = 3). Similar and Different letters represent groups that are not significantly and significantly different, respectively by ANOVA LSD.

32 µg/mL, and therefore sub-MIC concentration of the SeNPs, attained 40 percent and 55 percent respectively. This blend appeared to have a synergistic effect with 82% and 83% inhibition at MIC and sub-MIC respectively. On the contrary, TET with and without MIC acted with insignificant inhibition (~20%). The statistical analysis showed a lot of difference and combination treatment showed the highest inhibition. The results are indicative of the efficacy of SeNPs as biofilm-busting against Salmonella enterica, which were resistant to antibiotics. The improvement of effect in the case of SeNPs and TET can be discussed with the previous literature where the synergistic activity of nanoparticles and antibiotics was observed, disrupting bacterial membranes and enhancing the rate of antibiotic uptake [28,17]. Nanoparticles-based approaches are a promising strategy to combat biofilm-forming pathogens because of the moderate inhibition provided by TET alone.

CONCLUSION

In this study, the antimicrobial potential of biogenically produced selenium nanoparticles (SeNPs) with lemon peel extract by bio-reduction method was examined against clinical isolates of Salmonella enterica. Reliable detection was conducted by culturing, biochemical test, and molecularly based on confirmation of the identity of the isolates. There was a high rate of multidrug-resistant (MDR) strains susceptibility, which explains the necessity of an individual approach to treatment with constant monitoring of resistance. The SeNPs exhibited great antimicrobial effect with MIC of 32 µg/mL, which is in line with the reported results of plant-extract synthesized nanoparticles. Moreover, SeNPs demonstrated good antibiofilm activity and the formation of biofilm by Salmonella was suppressed by its combination with tetracycline, which indicated their potential use as supplement to classical antibiotics in prevention and treatment of biofilm-associated Salmonella infections. These results favor the application of biogenic SeNPs as effective antimicrobials, natural antioxidants, and low-toxicity options in dealing with bacteria resistant infections.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

REFERENCES

1. Bayda S, Adeel M, Tuccinardi T, Cordani M, Rizzolio F. The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. Molecules. 2019;25(1):112.
2. Karthik KK, Cheriyan BV, Rajeshkumar S, Gopalakrishnan M. A review on selenium nanoparticles and their biomedical applications. Biomedical Technology. 2024;6:61-74.
3. kazemi S, Hosseingholian A, Gohari SD, Feirahi F, Moammeri F, Mesbahian G, et al. Recent advances in green synthesized nanoparticles: from production to application. Materials Today Sustainability. 2023;24:100500.
4. Ansari JA, Malik JA, Ahmed S, Manzoor M, Ahemad N, Anwar S. Recent advances in the therapeutic applications of selenium nanoparticles. Mol Biol Rep. 2024;51(1).
5. Vignesh A, Menaka C, Amal TC, Selvakumar S, Vasanth K. Exploring the dual impact of nanoparticles on human well-being: A comprehensive review of risks and benefits. Next Nanotechnology. 2025;8:100223.
6. lawal H, Saeed SI, Gaddafi MS, Kamaruzzaman NF. Green Nanotechnology: Naturally Sourced Nanoparticles as Antibiofilm and Antivirulence Agents Against Infectious Diseases. Int J Microbiol. 2025;2025(1).
7. Yilan G, Cordella M, Morone P. Evaluating and managing the sustainability of investments in green and sustainable chemistry: An overview of sustainable finance approaches and tools. Current Opinion in Green and Sustainable Chemistry. 2022;36:100635.
8. Farré M, Pérez S, Gonçalves C, Alpendurada MF, Barceló D. Green analytical chemistry in the determination of organic pollutants in the aquatic environment. TrAC, Trends Anal Chem. 2010;29(11):1347-1362.
9. Lamichhane B, Mawad AMM, Saleh M, Kelley WG, Harrington PJ, Lovestad CW, et al. Salmonellosis: An Overview of Epidemiology, Pathogenesis, and Innovative Approaches to Mitigate the Antimicrobial Resistant Infections. Antibiotics. 2024;13(1):76.
10. Teklemariam AD, Al-Hindi RR, Albiheyri RS, Alharbi MG, Alghamdi MA, Filimban AAR, et al. Human Salmonellosis: A Continuous Global Threat in the Farm-to-Fork Food Safety Continuum. Foods. 2023;12(9):1756.
11. Nazir J, Manzoor T, Saleem A, Gani U, Bhat SS, Khan S, et al. Combatting Salmonella: a focus on antimicrobial resistance and the need for effective vaccination. BMC Infect Dis. 2025;25(1).
12. Mkangara M. Prevention and Control of Human Salmonella enterica Infections: An Implication in Food Safety. International Journal of Food Science. 2023;2023:1-26.
13. Rani S, Jahengir Alam K, Shankar Saha S, Rahman MM, Alam MS. Seroprevalence, Identification, and Pathology of Salmonellosis in Selected Poultry Farms at Barishal District of Bangladesh. Turkish Journal of Agriculture - Food Science and Technology. 2022;10(6):1158-1164.
14. Kipper D, Hellfeldt RM, De Carli S, Lehmann FKM, Fonseca ASK, Ikuta N, et al. Salmonella serotype assignment by sequencing analysis of intergenic regions of ribosomal RNA operons. Poult Sci. 2019;98(11):5989-5998.
15. An Overview of the Clinical and Laboratory Standards Institute (CLSI) and Its Impact on Antimicrobial Susceptibility Tests. Antimicrobial Susceptibility Testing Protocols: CRC Press; 2007. p. 15-20.
16. Stepanović S, Vuković D, Hola V, Bonaventura GD, Djukić

S, Ćirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007;115(8):891-899.
17. Salem SS, Badawy MSEM, Al-Askar AA, Arishi AA, Elkady FM, Hashem AH. Green Biosynthesis of Selenium Nanoparticles Using Orange Peel Waste: Characterization, Antibacterial and Antibiofilm Activities against Multidrug-Resistant Bacteria. Life. 2022;12(6):893.
18. Safaei M, Mozaffari HR, Moradpoor H, Imani MM, Sharifi R, Golshah A. Optimization of Green Synthesis of Selenium Nanoparticles and Evaluation of Their Antifungal Activity against Oral Candida albicans Infection. Advances in Materials Science and Engineering. 2022;2022:1-8.
19. Han H-W, Patel KD, Kwak J-H, Jun S-K, Jang T-S, Lee S-H, et al. Selenium Nanoparticles as Candidates for Antibacterial Substitutes and Supplements against Multidrug-Resistant Bacteria. Biomolecules. 2021;11(7):1028.
20. Basumatari M, Devi RR, Gupta MK, Gupta SK, Raul PK, Chatterjee S, et al. Musa balbisiana Colla pseudostem biowaste mediated zinc oxide nanoparticles: Their antibiofilm and antibacterial potentiality. Current Research in Green and Sustainable Chemistry. 2021;4:100048.
21. Mohammed AJ, Khadim MA, Hassoon SJ. Isolation and Identification of Salmonella Spp. Bacterial Pathogens from Frozen Chickens Meat in Diyala Province. Diyala Journal for Veterinary Sciences. 2023;1(2):61-68.
22. Neyaz LA, Alghamdi HS, Alghashmari RM, Alswat SS, Almaghrabi RO, Bazaid FS, et al. A comprehensive review on the current status of culture media for routine standardized isolation of Salmonella and Shigella spp. from contaminated food. Journal of Umm Al-Qura University for Applied Sciences. 2024.
23. Khadka P, Thapaliya J, Thapa S. Susceptibility pattern of Salmonella enterica against commonly prescribed antibiotics, to febrile-pediatric cases, in low-income countries. BMC Pediatr. 2021;21(1).
24. Hinson C, Tonouhewa A, Azokpota P, Daube G, Korsak N, Sessou P. Global prevalence and antibiotic resistance profiles of bacterial pathogens in table eggs: A systematic review and meta-analysis. Veterinary World. 2025:939-954.
25. Punchihewage-Don AJ, Ranaweera PN, Parveen S. Defense mechanisms of Salmonella against antibiotics: a review. Frontiers in Antibiotics. 2024;3.
26. Majumder MAA, Rahman S, Cohall D, Bharatha A, Singh K, Haque M, et al. Antimicrobial Stewardship: Fighting Antimicrobial Resistance and Protecting Global Public Health. Infection and Drug Resistance. 2020;Volume 13:4713-4738.
27. Musa L, Toppi V, Stefanetti V, Spata N, Rapi MC, Grilli G, et al. High Biofilm-Forming Multidrug-Resistant Salmonella Infantis Strains from the Poultry Production Chain. Antibiotics. 2024;13(7):595.
28. Effects of Thymus daenensis essential oil-loaded chitosan nanoparticles on BCR1 gene expression in Candida parapsilosis. Archives of Razi Institute. 2023:973-980.
29. Olaoye AB, Owoeye SS, Nwobegu JS. Facile green synthesis of plant-mediated selenium nanoparticles (SeNPs) using Moringa oleifera leaf and bark extract for targeting α-amylase and α-glucosidase enzymes in diabetes management. Hybrid Advances. 2024;7:100281.
30. Asaad AM, Saied SA, Torayah MM, Abu-Elsaad NI, Awad SM. Antibacterial activity of selenium nanoparticles/copper oxide (SeNPs/CuO) nanocomposite against some multi-drug resistant clinical pathogens. BMC Microbiol. 2025;25(1).
31. Yuan Q, Xiao R, Afolabi M, Bomma M, Xiao Z. Evaluation of Antibacterial Activity of Selenium Nanoparticles against Food-Borne Pathogens. Microorganisms. 2023;11(6):1519.
32. Sentkowska A, Konarska J, Szmytke J, Grudniak A. Herbal Polyphenols as Selenium Reducers in the Green Synthesis of Selenium Nanoparticles: Antibacterial and Antioxidant Capabilities of the Obtained SeNPs. Molecules. 2024;29(8):1686.
33. Ayad m, Al-Quhli S, Jassim M. Assessment of the Effect of Selenium Nanoparticles on Virulence Genes Expression csgD, adrA, and gcpA of Multi-Drug Resistant Salmonella enterica. Journal of University of Anbar for Pure Science. 2024;18(2):5-15.
34. Al-Roomi NM, Ajeel HH. Antibacterial Effect of Green Synthesis of Selenium Nanoparticles against Salmonella typhi in Small Buffalo. UTTAR PRADESH JOURNAL OF ZOOLOGY. 2024;45(5):80-90.