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Assessment of the Effect of Selenium Nanoparticles on the Expression of Virulence Genes *csgD, adrA*, and *gcpA* of Multidrug-Resistant *Salmonella enterica*

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ABSTRACT

This study investigated the antimicrobial properties of selenium nanoparticles (SeNPs) against the biofilm formation-related genes of multidrug-resistant (MDR) *Salmonella enterica* serovars, such as Typhi and Typhimurium. Clinical strains of these serovars were isolated. Their abilities to form biofilms were tested in a microtiter plate, and their resistance to different antibiotics were assessed. In all the isolates, the beta-lactamase activity was found to be an end level. Amoxicillin/clavulanic acid was the most effective antibiotic with 73.3% in vitro susceptibility. Resistance to tetracycline (66.7%), trimethoprim-sulfamethoxazole *S.* Typhimurium (48%) and *S.* Enteritidis (21.7%) dominated the serotypes. Most of the *Salmonella* isolates were capable of forming biofilms on plastic materials, thereby presenting the possibility for some levels of infection control and food safety issues to arise. The pattern of antibiotic resistance was surprisingly intricate, revealing the presence of cross resistance. Multiple antibiotic resistance against the commonly used antibiotics such as chloraophenolcicol, amoxicillin, and cefoxitin was also observed in the isolates from immunocompromised patients. Synergy was verified after the application of SeNPs combined with antimicrobials, and the antibiotic efficacy was enriched. In addition, SeNPs downregulated the expression of *adrA, gcpA*, and *csgD* genes encoding biofilm and acting as virulence markers. In the face of antibiotic resistance, SeNPs maybe eventually become a viable option among other strategies for persistent *Salmonella* infections. This study underscores the need for other techniques, particularly in the treatment of MDR *Salmonella*, and further inquiry as to the potential of SeNPs in the clinical field.

Introduction

Salmonella enteritidis is the most frequent cause of community-acquired bacterial sepsis, leading to considerable hospitalization and mortality cases primarily in immunocompromised individuals worldwide [1,2]. Patients with cancer are susceptible to severe infections, which may lead to invasive diseases and sepsis [1,3]. Even well-functioning intestinal tract lymphocytes usually have self-containing diarrheal illnesses. Risk factors for nontyphoid salmonella infections can include age, reduced gastric secretion, gastric surgery, antibiotic misuse, and some medications [4].

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With the increase in multidrug-resistant (MDR) *Salmonella* strains, treatment strategies have become complex and require measures such as biofilm prevention [5,6].

Certain virulence genes, such as *csgD, adrA*, and *gcpA*, play a crucial role in the biofilm formation of *Salmonella*. *CsgD* regulates the synthesis of biofilm matrix DNA and lipopolysaccharides and increases the pathogenicity level [7,8]. It is a transcriptional regulator that controls the expression of genes involved in synthesizing biofilm matrix components, such as extracellular DNA and lipopolysaccharides. Its overexpression enhances biofilm formation and increase the virulence of *Salmonella* in animal models of infection. *AdrA* and GcpA, both adhesins, are vital for

Salmonella's surface adhesion and biofilm formation [9].

Given the challenge of MDR bacteria, nanomaterials such as metal oxides offer alternative treatments. Nanoparticles (NPs) exhibit unique physical and chemical properties, particularly in medicine [10,11].

Selenium (Se), which is toxic at the nanoscale due to increased surface area and reactivity, is essential for human health as antioxidants in selenoproteins [12,13].

SeNPs are toxic against Gram-positive and Gram-negative bacteria, including MDR strains [14]. They have broad-spectrum toxicity to pathogenic bacteria but are nontoxic to humans and show effectiveness as antibacterial agents [18]. Their combination with conventional antibiotics offers enhanced antibacterial effectiveness, demonstrating synergistic and bactericidal properties [15,16].

Biofilm formation is a critical step in the pathogenesis of many bacterial infections, including those caused by MDR *Salmonella*. Biofilms provide a protective environment for bacteria, making them difficult to eradicate with conventional antibiotics [17].

Selenium nanoparticles (SeNPs) are extremely toxic to Gram-positive and Gram-negative bacteria, including multidrug-resistant bacteria. (SeNPs) are wide-spectrum toxic to pathogenic bacteria. For the human body, it was a non-toxic, effective antibacterial agent [18]. Selenium nanoparticles successfully combat Gram-negative and Gram-positive bacteria. A biological strategy combines nano-selenium with conventional antibiotics to increase their effectiveness against harmful microorganisms. Nano demonstrates synergistic and bactericidal properties. Nano has antibacterial properties as well as synergistic activity [14]

Materials and Methods

Samples

This study included 217 patients (aged 1–60 years) with clinical suspicion of *Salmonella enterica* infection, presenting symptoms such as diarrhea (gastroenteritis). Data were collected over a year (October 2022 to October 2023) from multiple healthcare facilities in Al-Ramadi. Inclusion criteria comprised symptoms such as diarrhea, stomach cramps, fever, nausea, vomiting, chills, headache, and blood in stool. Patients with a Bristol stool level of 6–7 and positive tests for calprotectin, transferrin, and occult blood were excluded.

Bacterial Isolation and Identification

Stool and blood samples were collected from immunocompromised and immunocompetent patients (Table 1).

Table1. Distribution of S. enterica cases according to type

	Immunocompromised	Immunocompetent	Total
Number of samples	89	128	217
Positive	48(57.8%)	35(42.16%)	83 (38.4%)
Negative	41(30.59%)	93(69.4%)	134(61.75%)
	89(100%)	128(100%)	

S. enterica serovars Typhi and Typhimurium were isolated and identified from clinical samples incubated for 18–24 hours at 37 °C on various media (s,s agar macConky XLD and blood agar). Colonies appeared as small, smooth, red with black centers on XLD agar, colorless on MAC, and nonhemolytic, white on blood agar. These characteristics confirmed the presence of *S.* Typhi and *S.* Typhimurium, with the XLD agar crucial for isolating *Salmonella*. Identification was further verified using the VITEK® 2compact system.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was tested using the Kirby–Bauer disk diffusion method on Muller–Hinton agar following CLSI2021 guidelines. Tested antibiotics included amikacin (AK), amoxicillin (AX), amoxicillin/clavulanic acid (AUG), levofloxacin (Lev), gentamicin (CN), ceftriaxone (CTR), cefotaxime (CTX), cefixime (Cf), ciprofloxacin (CIP), and chloramphenicol (C). *Escherichia coli* ATCC 25922 was used for quality control alongside clinical isolates [19].

Determination of Resistance

The Kirby–Bauer disk diffusion method used Muller-Hinton agar and various antibiotic discs.

Resistance was determined by measuring the growth inhibition zone diameter.

Minimum Inhibition Concentration calculated to S. enterica

The impact of AX, Lev, SeNPs, and the combination of SeNPs–AX on the gene expression of *S.* Typhi and *S.* Typhimurium at the SUB MIC level was studied using serial dilutions in microdilution methods and chequerboard methods. The SUB MIC considered 0.5 of the MIC effect.

Biofilm Formation

The biofilm formation by *S. enterica* was studied using the following detailed methodology:

1. Inoculum Preparation: Overnight cultures of bacterial isolates were adjusted to 0.5 McFarland standard in Tryptic Soy Broth (TSB) supplemented with 1% glucose to promote biofilm formation.
2. Microtiter Plate Assay: Seeding involved inoculating 200 µL of bacterial suspension into each well of a 96-well microtiter plate. The plate was incubated at 37 °C for 24 hours for biofilm development. Post-incubation, the wells were washed three times with PBS to remove nonadherent cells, and biofilms were fixed with 200 µL of 99% methanol.
3. Staining and Quantification: Crystal violet staining involved staining air-dried wells with 0.1% crystal violet for 15 minutes, followed by rinsing off excess stain. The bound dye was solubilized in 33% acetic acid. Biofilm biomass was quantified by measuring absorbance at 590 nm using a microplate reader, with statistical analysis comparing biofilm formation across strains and conditions.

Genetic Analysis

Polymerase chain reaction (PCR) was used to detect virulence genes (*adrA, csgD,* and *gcpA*). Primers were prepared following the manufacturer's instructions, and a DNA ladder was used for gene size verification. The process involved amplification, electrophoresis, and result interpretation.

PCR techniques were used to detect virulence and biofilm-related genes (*adrA, gcpA*, and *CsgD*) with primers from Microgen, South Korea**.**

Table 2: Primers used in this study

Primers		Sequences	product	annealing
16srRNA	F	CGGACGGGTGAGTAATGTCT	406	58
	R	GTTAGCCGGTGCTTCTTCTG		
adaA	F	TCGGGTGAGTCCTGAAAAAC	173	58
	R	ACGATCATCAGGAGGGTCAG		
csgD	F	ATTAACGGCGTGTTTTACGC	228	57
	R	CGACCTCGCGATTTCATTAT		
gcpA	F	CCAGCTCGGTTACAAGTCGT	203	58
	R	GATCGGCTATCCAGTTCAGG		
recA	F	GGGAACCTGAAACAGTCCAA	188	58
	R	GTTTCGCTACCCACGACATT		

Selenium Nanoparticles Synthesis

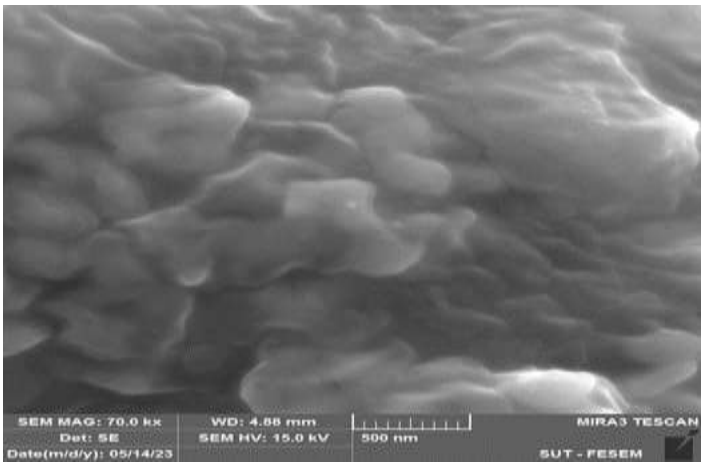
SeNPs were synthesized using a modified version of Vahdati's approach. In brief, 58.13 mM ascorbic acid (Merck, Germany) and 1.2 mM Na2SeO3 (Merck, Germany) were mixed in a 4:1 ratio and stirred at 1300 rpm at room temperature until the solution changed color from white to orange, signaling SeNP formation. Post-centrifugation at 12000 rpm, the pellet was washed and then resuspended in 1 mL of sterile double-distilled water with 30 µL of Tween 20 per 20 mL to avert aggregation during synthesis [14,20].

Characterization of selenium Nanoparticles

The analysis of SeNPs involved various methods: UV-vis spectrophotometer verified their identity within the 200–500 nm range (Perkin-Elmer, Thermo Scientific, USA). Size distribution and zeta potential were determined using a Zeta Sizer Nano Series -(Zetasizer Nano ZS, Malvern, Worcestershire, UK) post-sonication for 10 minutes. The Se content was assessed via ICP-AAS, employing a standard selenium concentration curve, followed by acid digestion using a 2% nitric acid solution for further scrutiny.

Structural groups were identified by FTIR analysis with a spectrometer (FTIR, PerkinElmer, USA) within the 400–4000 cm^{-1} wavenumber range. Morphology was assessed by scanning electron microscopy (FESEM, Tescan Mira3) and transmission electron microscopy

(TEM, Phillips EM 2085) at 100 kV. Image processing was performed using ImageJ software [21].



Picture1 SeNPs under Scanning Electron Microscopy (SEM)

Synergy Examination between Antibiotics and SeNPs

SeNPs were combined with antibiotics (AX) to enhance their bactericidal properties. Combination with Lev was also prepared to compare the nanomolecules with the highest and least resistance.

Determination of bactericidal action by SeNps

Bactericidal Efficacy Assessment: The antimicrobial activity of SeNPs was determined against a panel of pathogenic bacteria, including Gram-positive and Gram-negative strains. MIC and minimal bactericidal concentration (MBC) assays were performed to establish the effectiveness of SeNPs in inhibiting bacterial growth and causing bacterial death, respectively [22].

Additional experiments, including membrane permeability assays, intracellular reactive oxygen species (ROS) measurements, and DNA damage assessments, were performed to elucidate the underlying bactericidal action of SeNPs [23].

Statically analysis

Data were calculated by SPSS version 22 (IBM Corp. 2013, Armonk, NY) and analyzed by Chi-square test (cross tabulation) or Mann–Whitney test. All graphics (dot chart, bar chart, or scatter diagram) were generated with Microsoft Excel version 2016.

Results:

Confirmation of salmonella isolates by PCR amplification of16srRNA gene

PCR techniques targeting the 16srRNA genes *of S. enterica* serovars Typhi and Typhimurium were employed for sample analysis. Figure (1) illustrates the PCR method confirming *S. enterica* serovar Typhimurium identification via a 425 bp product within the 16srRNA gene. Vitek 2compact methods revealed that 63 samples contained this specific gene. This study underscores the utility of 16S rRNA genes in identifying the *Salmonella* genus and species, consistent with findings of Abdelaziz.

The antimicrobial activity of SeNps was determined against a panel of pathogenic bacteria, including both Gram-positive and Gram-negative strains. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assays were performed to establish the effectiveness of SeNps in inhibiting bacterial growth and causing bacterial death, respectively [22].To elucidate the underlying bactericidal action of SeNps, we performed additional experiments, including membrane permeability assays, intracellular reactive oxygen species (ROS) measurements, and DNA damage assessments [23].

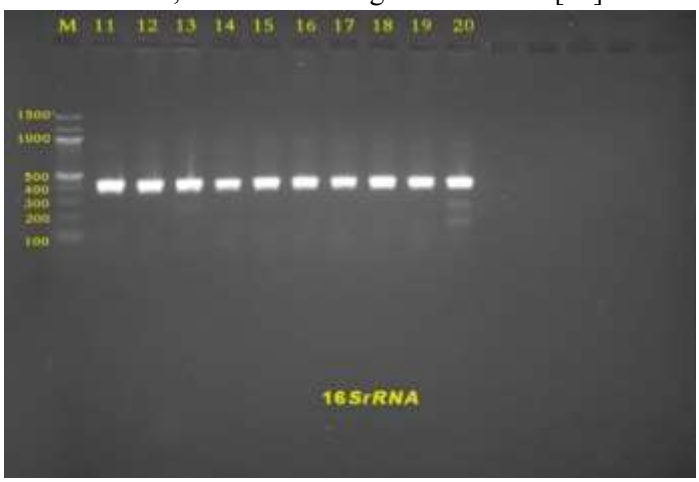


Figure1: Molecular identification of S. entrica isolates by 16srRNA gene with product 406, DNA Ladder(100bp), agarose conc. (1.5%) with Red Safe stain and electric current at 60 V for 85 min.

Molecular identification of virulence-associated genes

PCR analysis confirmed the presence of key virulence genes in all isolates of *S.* Typhi and *S.* Typhimurium. In particular, the production of 173 bp DNA fragments was confirmed, affirming the existence of the *adrA* gene. The presence of the *CsgD* gene was substantiated by the generation of 228 bp DNA fragments, and that of the *gcpA* gene was confirmed by the production of 203 bp DNA fragments. These findings collectively provided robust evidence of the presence of these virulence genes in our *Salmonella* isolates.

Detection of the virulence gene in the S. entries by PCR(adrA, csgD, and gcpA)

In this study, a result was positive when 70% of isolates of *S.* Typhi and *S.* Typhimurium produced 173 bp DNA fragments specific to the *adrA* gene.

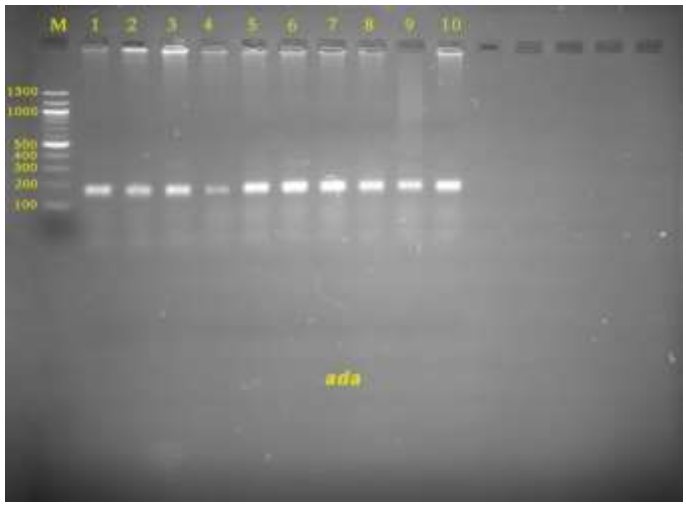


Figure 2: PCR amplification products of the *adrA* gene of *S. enterica* with product 173 bp, DNA ladder (100 bp), agarose conc. (1.5%) and red safe

All isolates of *S.* Typhi and *S.* Typhimurium produced 203 bp DNA fragments specific to the *CsgD* gene, indicating positive results. This result agrees with Seixas et al. (2014) and Raahati et al. (2020) as shown in Figure 4. More than 50% of the *S.* Typhi and *S.* Typhimurium isolates produced 173 bp DNA fragments specific to the *gcpA* gene, indicating their positive results (Figure 3).

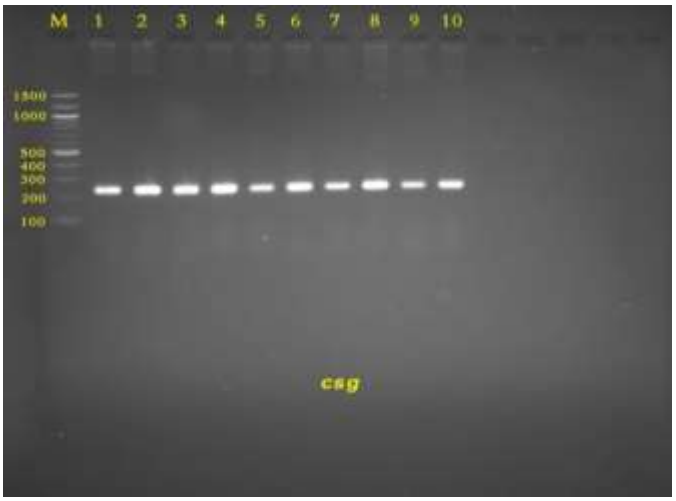


Figure 3: Amplification products of *CsgD* gene of *S. enterica* by PCR with product 203 bp, DNA ladder (100 bp), agarose conc. (1.5%) and Red-safe stain; electric current at 60 volts for 85 minutes.

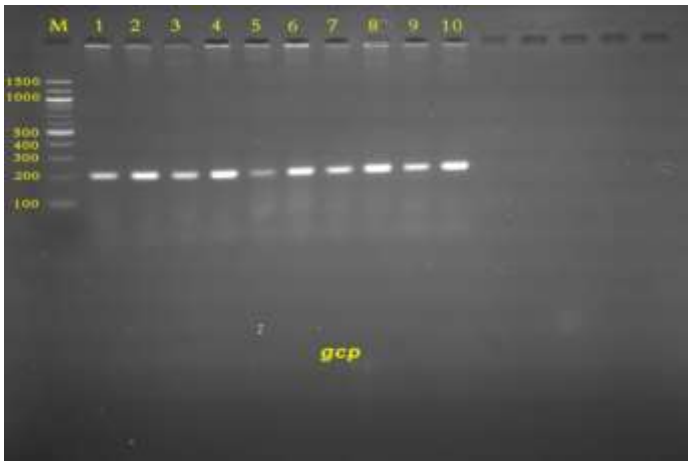


Figure 4: Amplification products of GCP gene of S. enterica by PCR with product 173bp, DNA Ladder(100bp), agarose conc. (1.5%) and red safe stain; electric current at 60 volts for 85 minutes.

Antibiotic susceptibility test of S. enterica in immunocompromised case

Figure 3 shows that 46/54 of *S. enterica* isolates (85.18%) are sensitive to levofloxacin, and all isolates (100%) are resistant to ampicillin, chloramphenicol, CTX, CTR, and Cf. Only 30/54 isolates (55.6%) are resistant to AUG, 29/54 isolates (53.7%) are sensitive to gentamycin, 30/54 isolates (55.6%) are sensitive to AK, and 31/54 isolates (57.4%) are sensitive to CIP.

Antibiotic susceptibility test of S. enterica in the immunocompetent patients group

Figure 2 shows that 19/26 of *S. typhi* isolates (73.1%) are sensitive to levofloxacin, 16/26 isolates (61.5 %) are sensitive to AK and CIP, and 17/26 isolates (65.5%) are sensitive to gentamycin. For the other antibiotics, the sensitivity of *S. typhi* isolates is low. Meanwhile, 18/26 isolates (69.23%) are resistant to chloramphenicol, 22/26 isolates (84.6%) are resistant to AX, 15/26 isolates (57.7%) are resistant to CTR, and 12/26 isolates (46.2%) are resistant to CTX. Other resistance and intermediate results are shown in **Table 3**. Significant differences were found in antibiotic sensitivity intermediate and resistance with P-value < 0.005.

Table 3. Comparison of antibiotic susceptibility in immunocompromised (ICD) and immunocompetent (ICT) cases for various antibiotics tested against *S. enterica*.

Antibiotics	Sensitive (%) ICD	Intermediate(%) ICD	Resistant (%) ICD	Sensitive (%) ICT	Intermediate(%) ICT	Resistant (%) ICT
Amoxicillin	**0.00**	**0.0%**	**100.0**	**19.23**	**23.1%**	**57.70**
Amikacin	**57.60**	**7.4%**	**35.1**	**61.50**	**26.9%**	**11.50**
Cefixime	**0.00**	**0.0%**	**100.0**	**40.60**	**31.3%**	**28.10**
Levofloxacin	**85.18**	**11.1%**	**3.7**	**73.10**	**19.23%**	**7.60**
Ampicillin	**0.00**	**0.0%**	**100.0**	**19.23**	**26.92%**	**53.80**
Gentamycin	**57.40**	**18.5%**	**24.1**	**65.50**	**30.8%**	**3.80**
Chloramphenicol	**0.00**	**0.0%**	**100.0**	**15.38**	**15.38%**	**69.23**
Ceftriaxone	**0.00**	**0.0%**	**100.0**	**23.07**	**19.23%**	**57.70**
Cefotaxime	**0.00**	**0.0%**	**100.0**	**11.50**	**42.3%**	**46.20**
Amoxicillin/Clavulanic	**55.60**	**24.1%**	**20.3**	**55.60**	**23.1%**	**20.30**

Selenium Nanoparticles and Their Antimicrobial Effects

Our findings revealed that SeNPs demonstrated significant bactericidal activity against various bacterial strains. The MIC and MBC values indicated that SeNPs effectively inhibited bacterial growth and induced bacterial death at low concentrations. Furthermore, mechanistic studies indicated that SeNPs disrupted bacterial cell membranes, increased intracellular ROS levels, and caused DNA damage, collectively contributing to their antimicrobial action.

Biofilm formation of *S. enterica* by microplate titer assay

The results of the microtiter experiment indicated that the isolates of *S. enterica* demonstrated a significant potential for producing biofilm on plastic surfaces. The isolates were categorized into groups based on their biofilm formation capacities. Out of the 80 clinical bacterial isolates of *S. enterica*, biofilm production was detected in 59 isolates (73.75%), indicating a high capacity to form biofilm on plastic surfaces. The remaining 21 isolates (26.25%) were classified as nonbiofilm producers with OD values below 0.15

Table4: Mean of OD value and biofilm formation [15].

Mean OD value	Biofilm formation
0.15<	**No biofilm**
0.15-0.24	**Weak biofilm**
0.25-0.39	**Moderate**
0.4	**Strong**

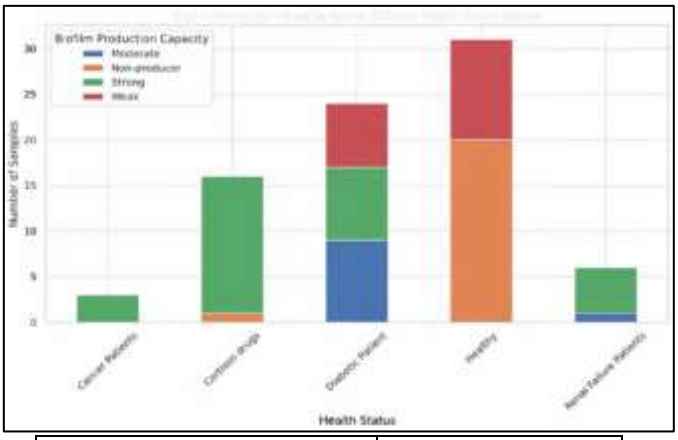


Figure5: Biofilm Production Capacity Across Different Groups.

Among the biofilm-producing *Salmonella* isolates, 35.59% showed strong biofilm formation, indicating robust adherence to microplate plastic surfaces. Moderate and weak biofilm formation was observed in 35.59% and 28.8% of isolates, respectively. This finding demonstrates the high capability of *Salmonella* to form biofilms on plastic, with implications for public health and food safety.

Real-Time- quantitative PCR (qPCR)

Extraction of RNA

RNA was extracted using a GENEzol TriRNA purification kit from four *S.* Typhi and *S.* Typhimurium isolates before and after treatment with sub-MIC of Lev, SeNPs alone, and a combination of SeNPs with AX. The concentration ranged between 118 and 232 ng/µL, and the purity fluctuated from 0.91 to 1.8.

The impact of AX, Lev, SeNPs, and a combination of SeNPs-AX on the gene expression of *S.* typhi and *S.* typhimurium were studied at the SUB-MIC level using serial microdilution and chequerboard methods. The SUB MIC considered 0.5 of the MIC effect.

In this study, we investigated the impact of various treatments on the expression of *adrA*, *gcpA*, and *csgD* in *S.* Typhimurium and *S.* Typhi. Our results revealed a notable reduction in the expression of these virulent genes under specific treatments. For *adrA* expression (Table 5) in *S.* Typhimurium, Lev was effective. In *S. Typhi*, Levo-SeNPs) significantly decreased its expression. (2−ΔΔCT) present the expression of the target gene, "Folding" refers to the fold change in gene expression (Folding > 1: Indicates upregulation, Folding < 1: Indicates downregulation and Folding = 1: Implies no change in gene expression) (Yuan Y.-G.2017).

Table5. *adrA* expression

Type of Bacteria	Treatment	$2^{-\Delta\Delta CT}$	Folding
S. Typhimurium	Se-NPs	0.222	4.5
	Amoxicillin	1.197	0.197
	Amoxi-SeNPs	1.443	0.443
	Levofloxacin	0.19	5.263
	Levo-SeNPs	0.189	5.291
	Control (without)	1	0
S. Typhi	Se-NPs	1.205	0.205
	Amoxicillin	1.443	0.443
	Amoxi-SeNPs	0.641	1.56
	Levofloxacin	0.381	2.624
	Levo-SeNPs	0.632	1.582
	Control (without)	1	0

Table6. *gcpA* expression

Type of Bacteria	Treatment	$2^{-\Delta\Delta CT}$	Folding
S. Typhimurium	Se-NPs	2.514	1.514
	Amoxicillin	4.856	3.856
	Amoxi-SeNPs	3.706	2.706
	Levofloxacin	1.815	0.815
	Levo-SeNPs	1.986	0.986
	Control (without)	1	0
S. Typhi	Se-NPs	1	0
	Amoxicillin	8.224	7.224
	Amoxi-SeNPs	0.582	1.718
	Levofloxacin	1.635	0.635
	Levo-SeNPs	1.624	0.624
	Control (without)	1	0

Regarding *gcpA* expression (Table 6), the combination of AX, SeNPs (Amoxi-SeNPs), and Lev induced a decrease in *S.* Typhimurium. In *S.* Typhi, Lev, particularly when combined with SeNPs (Levo-SeNPs), was effective in reducing *gcpA* expression. For *csgD* expression (Table 6) in *S.* Typhimurium, AX alone and its combination with SeNPs (Amoxi-SeNPs) effectively reduced its levels. For *S.* Typhi, SeNPs alone were sufficient to reduce *csgD* expression. These findings highlight the potential of these treatments, individually and in combination, in modulating the expression of key virulence genes in *Salmonella* species.

Table7. *csgD* expression

Type of Bacteria	Treatment	$2^{-\Delta\Delta CT}$	Folding
S. Typhimurium	Se-NPs	0.339	2.949
	Amoxicillin	11.551	10.551
	Amoxi-SeNPs	3.226	2.226
	Levofloxacin	0.248	4.032
	Levo-SeNPs	0.175	5.714
	Control (without)	1	0
S. Typhi	Se-NPs	3.41	2.41
	Amoxicillin	2.329	1.329
	Amoxi-SeNPs	0.959	1.042
	Levofloxacin	0.687	1.455
	Levo-SeNPs	0.795	1.257
	Control (without)	1	0

Discussion

The findings illuminate significant insights into the behavior of MDR *S. enterica* in immunocompromised and immunocompetent cases, particularly in the context of antibiotic susceptibility and biofilm formation. This study also highlights the newly developed SeNPs as a possible tool for modifying virulence gene expression in these bacteria.

The elevated level of multidrug resistance in the strains, especially in the immunocompromised patients, highlights the urgent requirement for the alternative treatment approaching. In particular, the application of SeNPs combined with antibiotics has remarkably improved the antibiotic's effectiveness while downregulating the expression of genes such as *adrA*, *gcpA*, and *csgD*, which are related to biofilm and virulence..

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Antibiotic Susceptibility

According to the results about the outer layer, most *Salmonella* isolates had multidrug resistance. This finding agreed with earlier studies, demonstrating that antibiotic resistance is a global problem among bacterial

pathogens[1,3,8].

Such preventions would be a huge challenge to the doctor–patient relationship, especially for immunocompromised patients who account for 75% of severe infections [24,25]. Patients become resistant to common antibiotics used in medicated treatments, such as chloramphenicol, AX, and CTR, stressing that the need for other approaches to healing. The antibiotic resistance of *S. enterica* and nanoparticles as a replacement therapy are in line with the current research. [26] underscored the significance of nanotechnology in raising crop productivity and combating bacterial resistance that deals directly with our sprinkling area. The findings of Khurana et al. (2019) and Ferro et al. (2021) on the therapeutic effects of SeNPs strengthened our antimicrobial outcomes.

Biofilm Formation

The analysis of biofilm formation is undoubtedly an important one. In line with previous works showing biofilm formation as an important factor of virulence [4,26], *S. enterica* built deep biofilms on plastic surfaces in the microtiter plate assay. The formation of biofilms is directly correlated to the persistence of a bacterial infection and the resistance to antibiotics, hence the worsening of treatment strategies [11].

Gene Expressions

The down regulation of *adrA*, *gcpA*, and *csgD* by different types of treatment shows different types of antibacterial activity and effectiveness of these compounds against *Salmonella* strains.

Lev in combination with SeNPs showed a superior efficacy in lowering *adrA* and *gcpA* expression in *S.* Typhimurium and *S.* Typhi, suggesting that this antibacterial/agent affected the cell wall formation or blocked the DNA replication. AX, especially when combined with SeNPs, effectively repressed *gcpA* and *csgD* expressions in *S.* Typhimurium, implying that it can target key virulence factors. The ability of SeNPs alone to reduce *csgD* expression in *S.* Typhi also indicates its potential as a single agent and in combination with other antibiotics.

This study demonstrates unequivocal evidence that Lev and SeNPs considerably decrease the expression of the main virulence genes of *S.* Typhimurium and *S.* typhi. This finding is important for MDR bacterial infections where the older antibiotic do not work anymore. The synergy between SeNPs and antibiotics such as AX opens up new paths of antimicrobial treatment that is effective against drug-resistant strains [2,6].

Role of Selenium Nanoparticles

This delineates SeNPs as a novel strategy fighting MDR *Salmonella.* SeNPs exhibited extraordinary antimicrobial properties, actively affecting h Gram-positive and Gram-negative bacteria. This ability even extends to MDR strains [13,14,25]. When administered together with certain antibiotics, SeNPs suppressed the growth and eventually killed the bacteria in some of the cases, increasing the effectiveness of the antibiotics.

The use of SeNPs as a possible gene modulator of virulence gene expression is a new innovation. SeNPs have demonstrated their powerful activity against many bacteria, including Gram-positive, Gram-negative, and MDR strains [13,16,27].

The effectiveness of the combined use of antibiotics and SeNPs indicate their synergistic effect. Such phenomenon can be achieved when SeNPs destroy the bacterial cell walls and membranes, enhance the penetration of antibiotics, or even interfere with the genes related to virulence and biofilm formation [28].

Methodological Considerations

This strength of our approach lies in the complete examination of biofilm formation, antibiotic resistance patterns and SeNP synthesis. Nevertheless, the study limitation lies in its focus on the specific serovars of *S. enterica* and only inactivated *S.* Typhimurium. Future research should conduct serovar-specific analysis to clarify the nature of the pathogen.

Conclusion

This research highlights SeNPs as regulatory factors to lower the virulence gene expression in MDR *S. enterica* serovars Typhi and Typhimurium. Our data displayed a dramatic reduction in virulence genes of both S. enterica serovars. Such influence is reported in the cases of immunodeficiency and people with a functioning immune system, illustrating the capacity of SeNPs to prove their effectiveness in such a wide range of situations. The experiment shows the strong point of

SeNPs in terms of overcoming antibiotic resistance as alternative or add on therapy to traditional antibiotics used in disease control

LIMITATIONS OF THE STUDY

This study focused exclusively on two *S. enterica* serovars, namely, Typhi and Typhimurium. This limitation restricts the generalizability of the findings to other serovars that may exhibit different responses SeNPs. In addition, the study involved patients from specific healthcare facilities in Al-Ramadi City, Iraq, which might not represent a diverse patient population.

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تقييم تأثير جسيمات السيلينيوم النانوية على تعبير جينات الفوعة csgD, adrA, gcpA لبكتيريا السالمونيلا المعوية المقاومة للأدوية المتعددة

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الخلاصة:

تستكشف هذه الدراسة استخدام جسيمات السيلينيوم النانوية (SeNPs) لمكافحة سلالات السالمونيلا المعوية المقاومة للأدوية المتعددة (MDR)، مع التركيز على تكوين الأغشية الحيوية وأنماط مقاومة المضادات الحيوية. تضمن البحث عزل سلالات السالمونيلا المصلية من العينات السريرية، وتقييم قدراتها على تكوين الأغشية الحيوية باستخدام اختبار Microtiter plate assay، وتحليل مقاومتها للمضادات الحيوية المختلفة. من النتائج المهمة لهذه الدراسة هو الميل العالي لعزلات السالمونيلا لتكوين أغشية حيوية على الأسطح البلاستيكية، مما يثير المخاوف بشأن مكافحة العدوى وسلامة الأغذية. كشفت اختبارات الحساسية للمضادات الحيوية عن مستوى ينذر بالخطر من مقاومة الأدوية المتعددة، وخاصة في العزلات من المرضى الذين يعانون من ضعف المناعة. وكانت هذه المقاومة واضحة ضد المضادات الحيوية في الخطوط الأمامية مثل الكلورامفينيكول والأموكسيسيلين والسيفترياكسون. والجدير بالذكر أن الدراسة توضح أن SeNPs، عند دمجها مع المضادات الحيوية، تعمل على تحسين فعاليتها بشكل كبير. يتجلى هذا التعزيز بشكل خاص في انخفاض التعبير عن الجينات المرتبطة بالأغشية الحيوية والفوعة مثل adra و gCPA و esgD. يمكن أن تكون SeNPs طريقة واعدة في علاج عدوى السالمونيلا المقاومة، مما يوفر حلاً محتملاً لمشكلة مقاومة المضادات الحيوية المتصاعدة. بشكل عام، تسلط الدراسة الضوء على الحاجة الملحة لعلاجات بديلة ضد السالمونيلا المقاومة للأدوية المتعددة، وتشير إلى أن SeNPs قد يكون خيارًا فعالاً، مما يستدعي إجراء المزيد من الأبحاث للتطبيق السريري.