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PREPARATION, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY OF NEWLY BIOSYNTHESIZED AMPICILLIN/CHITOSAN/SELENIUM NANOCOMPOSITE (AMP/CS/SENC) USING *FUSARIUM FUJIKUROI* PP794203 AGAINST MULTIDRUG-RESISTANT *ESCHERICHIA COLI* PP797596

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ABSTRACT

Nowadays, nanobiotechnology is being used to restrict the development of bacterial resistance, particularly multidrug-resistant strains. Applications for selenium nanoparticles (Se NPs) are becoming progressively more widespread due to their broad bioactivity and antibacterial action, although few studies report their use as nanoparticles (NPs) in combination with antibiotics. The present study evaluated the combined effects of ampicillin (AMP), chitosan (CS) and Se NPs (AMP/CS/SeNC) against the multidrug-resistant *Escherichia coli* PP797596. Se NPs were biosynthesized using *Fusarium fujikuroi* (PP794203) in a green, safe, and fast method. AMP/CS/SeNC was characterized by UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Zeta potential, and transmission electron microscope (TEM) analyses. AMP/CS/SeNC revealed a characteristic peak at 280 nm. FTIR confirmed the presence of proteins as stabilizing and capping agents. Zeta potential refers to the high stability of AMP/CS/SeNC due to an intensive positive net surface charge (+44.3 mV). AMP/CS/SeNC appeared as crystalline-shaped NPs with an average particle size of 85.32 ± 1.8 nm. AMP/CS/SeNC showed strong antibacterial activity against the tested *E. coli* and displayed an inhibition zone of 21 ± 0.06 mm and a significantly minimum inhibitory concentration of 25 µg/ml. In addition, the prepared nanocomposite completely destroyed the tested *E. coli* cells, including the cytoplasmic membrane and cell wall, according to TEM studies. The current study provides a new nanocomposite that might be applied to different pharmaceutical products and an exceptional antibacterial potential against multidrug-resistant bacteria.

Keywords: Selenium, nanocomposite, biosynthesis, antibacterial activity, *Fusarium fujikuroi*, *Escherichia coli*, bacterial resistance

INTRODUCTION

Multidrug-resistant bacteria are a growing problem that causes a lot of mortality annually. These bacteria have become resistant to the available antibiotics as a result of improper overuse and have therefore gained a lot of attention to develop or replace these drugs with safe and more effective alternative remedies. This problem is now described and known as the antimicrobial resistance (AMR) crisis (**Abu-Elghait *et al*., 2021; Hansson & Brenthel, 2022; Puvača & Frutos, 2021**). On the other hand, many different bacteria and basic eukaryotes frequently develop into surface-associated masses known as biofilms **(Mohamed *et al*., 2021)**. Since it acts as a barrier to stop the entry of host defenses and antimicrobial medications into pathogenic cells, microbial biofilm is a critical component in many microbial diseases **(Abdelhameed *et al*., 2020; Abu-Elghait *et al*., 2021; Hamed *et al*., 2021)**. The discovery of a wide variety of antimicrobial compounds and the effective use of penicillin have resulted in the golden era of antibiotics **(Ribeiro da Cunha *et al*., 2019)**. *Escherichia coli* is a typically resistant β-lactam bacterium like cephalosporin and penicillin (oxacillin and methicillin) **(Medeiros *et al*., 1974)**. About 20% - 45% of multidrug-resistant *E. coli* is resistant to first-line antibiotics, such as trimethoprim-sulfamethoxazole, cephalosporins, and fluoroquinolones as reported in several epidemiological studies conducted in Europe, North America, and South America in the 2000s (**Foxman, 2010**). Due to resistance to these medications, proper therapy is being delayed, which raises morbidity and death rates **(Pitout, 2012)**. The carbapenems, temocillin, pivmecillinam, tigecycline, and colistin are usually recognized as recommended drugs for the treatment of chronic infections with ampicillin (AMP)- and extended-spectrum β-lactamases (ESBLs)-producing *E. coli* **(Pitout, 2010)**. However, due to the possibility of resistance, these antibiotics should only be used in cases where there are no other options. Designing a new antibacterial agent is essential to combating bacterial resistance to available antibiotics **(Nguyen *et al*., 2017)**.

Nanotechnology is concerned with developing and manipulating materials with a size range of 1-100 nm **(Salem & Fouda, 2021)**. Notably, optical, electrical, and magnetic characteristics of nanomaterials are significantly influenced by their shape and size, mostly owing to the high surface area-to-volume ratio **(Benelli *et al*., 2018)**. Currently, nanomaterials are employed in a variety of fields, such as veterinary, pharmaceutical, agricultural, and medical sciences **(Thamilarasan *et al*., 2018)**, sol-gel **(Bai *et al*., 2011)**, photochemical reduction **(Nath *et al*., 2004)**, physical and chemical vapor deposition **(Ren *et al*., 2004; Wang *et al*., 2007)**, mechano-sonochemical techniques **(Khubulava, 2018)**, and other top-down and bottom-up synthesizing techniques, have all been employed effectively to date in order to create novel nanoscale particles of various sizes and shapes. However, the harmful limitations are brought on by the costly and risky substances frequently utilized in these synthesizing techniques **(Saini & Ledwani, 2022)**. The recently emerged field of "biological synthesis" is the result of the productive combination of the various aspects of nanotechnology and biotechnology. In this developing field, microorganisms like bacteria, fungi, yeasts, and plants were used to synthesize biogenic nanoparticles (NPs) either intracellularly or extracellularly, where biomolecules function as reducing and stabilizing agents **(Boroumand Moghaddam *et al*., 2015)**. Intracellular approaches necessitate more time- and money-consuming extraction procedures as well as additional purification steps **(Nguyen *et al*., 2023; Singh *et al*., 2020)**. Numerous benefits come with the fungus-mediated green NPs formation, including straightforward scaling up, simple processing, viability from an economic standpoint, processing of biomass, and recovery of significant surface distances with the best mycelia expansion **(Wadhwani *et al*., 2016)**. Moreover, fungi secrete higher amounts of proteins than other microorganisms that act as reducing agents for NPs synthesis **(Anil Kumar *et al*., 2007)**. NPs synthesis is achieved by the bioreduction of bulk materials to their elemental forms, which is induced by functional groups like amines and alkanes that are extensively present in metabolites such as flavonoids, tannins, alkaloids, steroids, and terpenoids **(El-Zahed *et al*., 2023; Menon *et al*., 2019)**.

Selenium (Se) is an essential trace element that is known as being important for the metabolism of thyroid hormones and other vital metabolic processes in the human body. Because of its connection to the immune system and potential to cure cancer, Se is frequently utilized as a dietary supplement **(Fairweather-Tait *et al*., 2010; Liu *et al*., 2009)**. Proteins and Se were joined to generate seleno-proteins, which are crucial as antioxidant catalysts **(Abdelaleem *et al*., 2016)**. Additionally, Se was also discovered to be a part of detoxifying enzymes like glutathione peroxidase and thioredoxin reductase **(Okuno *et al*., 2001; Zhang *et al*., 2005)**, which remove heavy metals from the body, based on the electronic properties of Se ions **(Menazea, 2020; Youness *et al*., 2018)**. At the nanoscale, particles have a greater surface-to-volume ratio, which exposes more of their surface, enhancing

Se activity more deeply in the nano-regime. It lacks can harm the liver, heart, kidneys, skeletal muscle, and testes **(Wang *et al*., 2013)**, as well as cause cardiovascular problems and prostate cancer, whereas long-term Se supplementation or an excess of Se dosages exceeding 400 g/day is very hazardous to living cells **(Kristal *et al*., 2014; Rayman *et al*., 2018; Vinceti *et al*., 2018)**. In order to decrease the toxicity of inorganic Se in the case of living things, selenite and selenate ions are reduced to Se NPs **(Cepoi *et al*., 2023)**. Se nanoparticles (Se NPs) may be produced by a variety of microorganisms, according to many reports **(Singh *et al*., 2016; Srivastava & Mukhopadhyay, 2015; Wadhwani *et al*., 2016)**. It was reported that the most popular microorganisms for the production of Se NPs were bacteria **(Presentato *et al*., 2018; Pyrzynska & Sentkowska, 2022; Shakibaie *et al*., 2010)** and fungi **(Bafghi *et al*., 2021; Diko *et al*., 2020; Srivastava & Mukhopadhyay, 2015; Zhang *et al*., 2019)**. Only five fungi have been shown to be capable of producing Se NPs, namely *Aspergillus terreus* **(Zare *et al*., 2013)**, *Alternaria alternata* **(Sarkar *et al*., 2011)**, *Lentinula edodes* **(Vetchinkina *et al*., 2013)**, *Fusarium* sp., and *Trichoderma reesei* **(Gharieb *et al*., 1995)**. Se NPs demonstrated antimicrobial action against common and antibiotic-resistant phenotypes of both Gram-positive and Gram-negative bacteria **(Geoffrion *et al*., 2020)**, *Candida albicans* **(Kheradmand *et al*., 2014)**, *Trichophyton rubrum* **(Yip *et al*., 2014)**, *Pseudomonas aeruginosa*, and *Proteus mirabilis* **(Shakibaie *et al*., 2015)**.

Chitosan (CS) is a natural polymer that possesses antimicrobial potential against a variety of pathogens, including bacteria, fungi, and yeasts **(Kong *et al*., 2010; Tayel *et al*., 2010; Tayel *et al*., 2011)**. Two forms of chitosan (solution and film) possessed antibacterial properties **(Islam *et al*., 1970; Shameli *et al*., 2011).** Because CS film has poor antibacterial activity, it must be combined with other antibacterial materials in order to be used in film-based products. CS can be combined with NPs of metal to improve its antibacterial action **(Wei *et al*., 2009)**. The antibacterial **(Raut *et al*., 2016)**, antifungal **(Sathiyabama & Parthasarathy, 2016)**, antibiofilm **(Costa *et al*., 2017)** and anticancer **(Subhapradha & Shanmugam, 2017)** characteristics of CS combined with metal and metal oxide NPs are significant. They may also be utilized as biosensors **(Kaushik *et al*., 2008)**, nanofertilizers **(Duhan *et al*., 2017)**, eliciting agents **(Chandra *et al*., 2015)**, and pesticides (**Gabriel Paulraj *et al*., 2017; Murugan *et al*., 2016, 2017)**.

In this study, an innovative amalgamation of biogenic Se NPs produced by *Fusarium fujikuroi* combined with AMP and CS was prepared and examined for their antibacterial activity *in vitro* against multidrug-resistant *E. coli*.

MATERIALS AND METHODS

Microbial strains and culture conditions

Bacterial strain *E. coli* (AC: PP797596) and fungal strain *F. fujikuroi* (AC: PP794203) were obtained from the Microbiology Lab, Botany and Microbiology Department, Faculty of Science, Damietta University, Egypt.

Green synthesis of Se NPs

The biological preparation of Se NPs was carried out according to **Islam *et al*. (2022)** with some modifications. Briefly, 250 ml of malt extract glucose yeast extract peptone (MGYP) broth medium **(Abbas & Abou Baker, 2020)**, 0.03 M sodium selenite (Na_2SeO_3), 4 discs (5 mm) of *F. fujikuroi* were mixed, adjusted to pH 6, incubated at 30 ±2°C for 2-3 days at 150 rpm until the appearance of a red color, which indicated the Se NPs formation. Then, the suspension was collected by centrifugation at 5000 rpm for 10 min., and the residue was washed three times with distilled H_2O to eradicate excess impurities, then centrifuged and dried.

Synthesis of ampicillin/chitosan/Se nanocomposite

An aliquot of 30 mg of Se NPs was dispersed in 10 ml of distilled H_2O and ultrasonicated for 15 min by using an ultrasonic bath (Elmasonic S100H, Germany, 50/60 Hz). 30 mg chitosan (MW 50–190 KDa, deacetylation degree: ≥85%, Sigma-Aldrich, USA) was prepared in 2.5% acetic acid (pH 8), mixed with a solution of Se NPs in a ratio of 1:1 v/v at 25°C. Then, 30 mg of antibiotic was added to the reaction mixture and stirred at 600 rpm using a magnetic stirrer/hot plate (Stuart UC152, UK) for 15 min. to obtain a transparent solution. After 6-8 h., drug-capped loading NPs were centrifuged at 5000 rpm and the residue was rinsed three times with distilled H_2O. The supernatant was neglected, and the drug-capped NPs residue was dried in an oven at 40-50°C. The final product was kept for the next step.

Characterization studies

The spectrum of ampicillin/chitosan/Se nanocomposite (AMP/CS/SeNC) was detected by using UV-Vis spectrophotometry (Double beam spectrum UV–Vis spectrophotometer V-760, JASCO, UK), X-ray diffractometer (XRD, X-ray diffractometer, model LabX XRD-6000, Shimadzu, Japan), and Fourier transform infrared spectroscopy (FT/IR-4100typeA). AMP/CS/SeNC was analyzed using Malvern Zetasizer Nano-ZS90 (Malvern, UK), and transmission electron microscopy (TEM, JEM-2100, Japan).

Antibacterial activity using agar well diffusion method

The antibacterial action of tested compounds was investigated according to the Clinical Laboratory Standards Institute **(CLSI, 2017)**. 100 µl of tested bacterial suspension (2.5×10^8 CFU.ml^{-1}) was added to cold melted Mueller-Hinton agar (MHA) medium (Oxoid, UK) at the time of pouring the plates in triplicate. After solidifying the agar plates, 100 µl of a unified concentration (150 µg/ml) of CS, AMP, Se NPs, and AMP/CS/SeNC was added separately into punched holes (5 mm) under aseptic conditions and then incubated at 37°C for 24 h. Then, the inhibition zones of bacterial growths were measured in mm.

Minimum inhibitory concentration (MIC)

The MIC tests of the tested compounds against treated bacterial strains were evaluated according to **CLSI (2000)**. Different concentrations (1-200 µg/ml) of CS, Se NPs and AMP/CS/SeNC were added into Mueller-Hinton broth (MHB) medium flasks inoculated by 200 µl (l2.5 × 10^8 CFU.ml^{-1}) tested bacteria and incubated at 37°C and 150 rpm for 24 h. Untreated bacteria and penicillin-treated bacteria were used as controls. Bacterial growth rates were measured spectrophotometrically (λ: 600 nm) against controls.

Ultrastructural analysis of AMP/CS/SeNC-treated *E. coli*

The bacterial cell cultures were exposed to AMP/CS/SeNC (MIC value) for 2 h at 37°C in MHB. Bacterial cells were washed, fixed with 2.5% glutaraldehyde and 0.1 M cacodylate buffer, pH 7, and sent to the Central Laboratory, Electron Microscope Unit, Faculty of Agriculture, Mansoura University, Egypt, for observing and studying their ultrastructure. Upon the removal of the fixative, 0.1 M buffer was introduced for washing, and the sample was afterward fixed for 90 min using 2% osmium tetroxide. A graded series of ethanol was used to dehydrate the fixed cells. After being dried, the cells were immersed in a 1:1 combination of Epon-Araldite for 1 h, and then the mixture polymerized for 24 h at 65°C. The cells were sectioned using an ultra-microtome (50 µm), double-stained with lead citrate and uranyl acetate, and then seen on carbon-coated copper grids (Type G 200, 3.05 µM diameter, TAAP, U.S.A.) using a TEM (JEOL JEM-2100, Japan) **(El Zahed *et al*., 2024)**.

Statistical analysis

The data were tested using the ANOVA test and SPSS software version 18. The significance level was established at 0.05. All experiments were done in triplicate. Each result was displayed together with its standard deviation (SD) and mean.

RESULTS AND DISCUSSION

Synthesis and characterization of AMP/CS/SeNC

Biosystems aimed to use microorganisms in different biotechnology applications including nanomaterial production **(Mohamed & El-Zahed, 2024)**. In a biosystem, different microorganisms including fungi, bacteria and algae are used as biocatalysts to convert bulk matter into nanoscaled materials. The use of different fungi in biosystem is common and can be beneficial because they can break down complex organic matter that other microorganisms cannot, thus increasing the efficiency of the system. *Fusarium* sp. is known to produce various secondary metabolites, including carotenoids that act as strong bioreducing agents **(Gharieb *et al*., 1995)**. The current study demonstrated the use of *F. fujikuroi* as a new nano-factory for Se NPs. Se NPs biosynthesis may be conducted in two main ways: employing living cells or various cell extracts. In both situations, the creation of NPs is dependent on the matrix's reduction potential. Two key enzymes, selenate reductase and selenite reductase, are responsible for the intracellular synthesis of Se NPs, whereas other chemical substances with reducing potential are responsible for extracellular production **(Afzal & Fatma, 2021)**. Due to the biomolecules' natural covering, biogenic Se NPs are more stable and do not agglomerate **(Wadhwani *et al*., 2016)**.

The synthesis of Se NPs was initiated when the colorless solution changed to a red-orange color known as a "brick" (Figure 1). In the present study, the synthesis of Se NPs was confirmed by the Surface Plasmon Resonance feature (SPR), which is illustrated by their unique λ_{max} at 252 nm in the UV-visible spectrum, indicating a good dispersion of particles in the noncolloidal (Figure 1). Our result is consistent with the findings of **Abbas & Abou Baker (2020) and Islam *et al*. (2022)** who confirmed the absorption peaks of Se NPs appeared in the range of 240-270 nm. The maximum absorbance of AMP/CS/SeNC colloidal suspensions was shifted to 280 nm which might be due to the binding with CS and AMP **(Nasir *et al*., 2017)**.

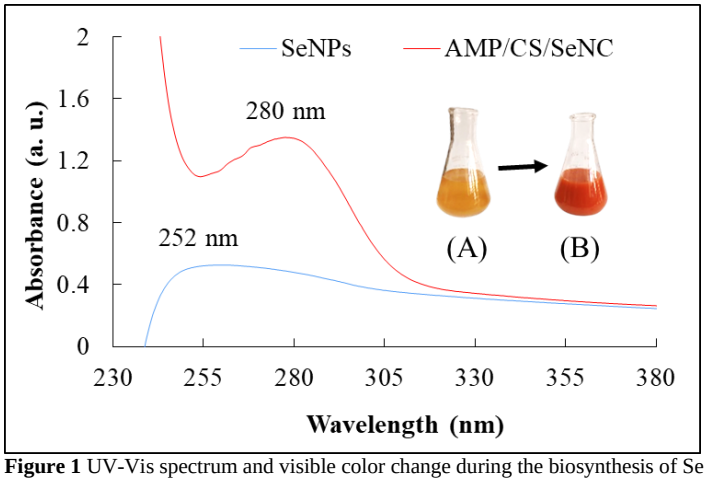

Figure 1 UV-Vis spectrum and visible color change during the biosynthesis of Se NPs and AMP/CS/SeNC. (A) Color of the reaction mixture at the beginning of experiment. (B) Color of the reaction mixture after the incubation time.

The crystallographic structure of the mycosynthesized AMP/CS/SeNC was determined using XRD studies (Figure 2). The diffraction patterns of AMP/CS/SeNC showed sharp and intense peaks at 2θ angles of 23.64°, 29.8°, 34.48°, 43.73°, 45.38°, 59.23°, and 66.2° which were indexed as 100, 101, 222, 102, 111, 112 and 210 planes, respectively, that correspond to the standard JCPDS data (JCPDS No. 06-0362). Moreover, the Scherrer equation was utilized to determine the crystallite size of AMP/CS/SeNC based on the FWHM of the most potent peak, which was 85.32 ± 1.8 nm.

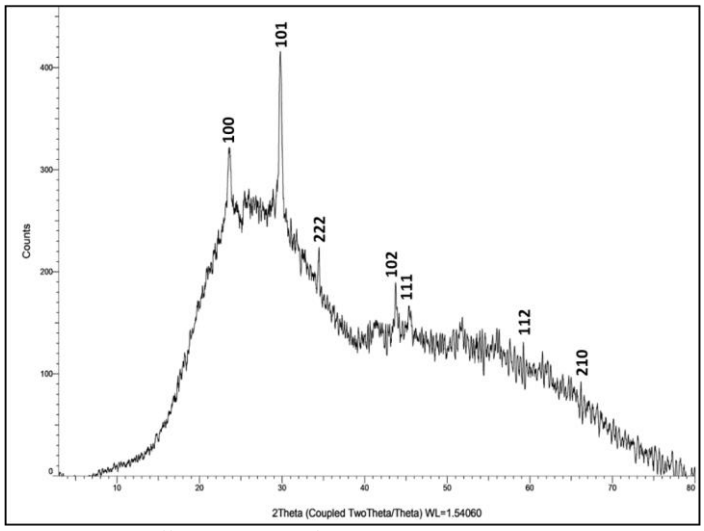

Figure 2 X-ray diffraction pattern of AMP/CS/SeNC.

The AMP/CS/SeNC and Se NPs FTIR spectra for this investigation (Figure 3) matched the **Gharieb *et al*. (2023) and Sonkusre *et al*. (2014)** results. The emergence of significant broad peaks around 3429 cm^{-1} (Se NPs) and 3387 cm^{-1} (AMP/CS/SeNC) indicates hydroxyl (O–H) groups and N–H stretching. Moreover, the FTIR spectra showed bands at 2937 cm^{-1} (Se NPs) and 2993 cm^{-1} (AMP/CS/SeNC) which indicated C–H stretching. The peaks at 2358 cm^{-1} (Se NPs) and 2632 & 541 cm^{-1} (AMP/CS/SeNC) are observed and correlate to the presence of proteins. Bands at 1645cm^{-1} (Se NPs) and 1733 cm^{-1} (AMP/CS/SeNC) were associated with proteins' amide I (N–C=O-stretching mode). The more complex amide band is located close to 1566 &1410 cm^{-1} (Se NPs) and 1459 & 1376 cm^{-1} (AMP/CS/SeNC) correspond to amide II (N–H bending mode) and amide III. The stabilization of metal ions and the synthesis of reduction were carried out by the amide groups, which indicated the existence of enzymes **(Prasad & Selvaraj, 2014)**. According to **Díaz-Visurraga *et al*. (2012)**, the finding suggests that the NPs are linked to molecules containing these functional groups. Therefore, based on this data, it is possible that the proteins created a capping agent on top of the Se NPs, which may have contributed to their stability. The C–O stretching mode might be the cause of the bands 1245 & 1085 cm^{-1} (Se NPs) and 1224, 1127 & 1045 cm^{-1} (AMP/CS/SeNC) **(Khiralla & El-Deeb, 2015)**. Se stretching vibration (C–Se) explains the appearance of 606 cm^{-1} (Se NPs) and 640 cm^{-1} (AMP/CS/SeNC). Our results were confirmed by **Qian *et al*. (2017),** who showed that the peak at 493.7 cm^{-1} is attributed to the Se-Se vibration. Other Se-related bonds are identified at C-Se (611.8 cm^{-1}).

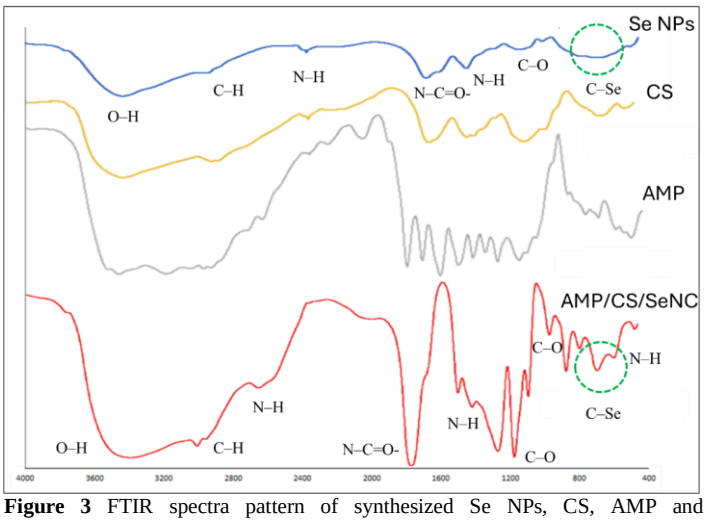

Figure 3 FTIR spectra pattern of synthesized Se NPs, CS, AMP and AMP/CS/SeNC.

Nanomaterials' impacts on medical applications are mostly determined by their size and stability. Thus, zeta potential must be used to investigate the stability and size distribution of the NPs. Colloid stability is significantly influenced by the surface charge, which may be studied using zeta potential data; a comparatively low zeta potential may be indicative of nanoparticle agglomeration. Being significantly positively charged or negatively charged is correlated with a potential of greater than +30 mV or less than -30 mV, respectively **(Lowry *et al*., 2016; Marsalek, 2014)**. In the present study, an intensive positive net surface charge at +44.3 mV was observed, so this is evidence of the high stability of AMP/CS/SeNC (Figure 4). On the other hand, previous studies recorded the negative charge of the biosynthesized Se NPs which was around -20 mV as reported by **Hussein *et al.* (2022),** who used different endophytic fungi for Se NPs biosynthesis, including *A. quadrilineatus*, *A. ochraceus*, *A. terreus*, and *F. equiseti*.

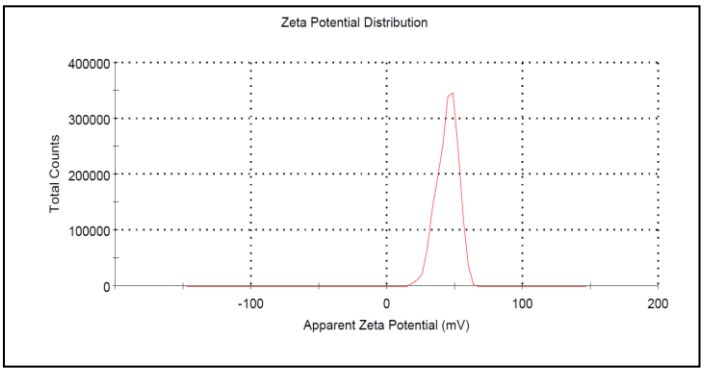

Figure 4 Zeta potential of AMP/CS/SeNC.

The TEM method is an imperative instrument to evaluate the size and shape of the produced NPs. TEM images clearly show the crystalline and uniformly distributed NPs varying from 80 nm to 90 nm in diameter, which matches the Scherrer equation results (Figure 5). According to **Gharieb *et al*. (2023)** the size of individual synthesized Se NPs by *F. oxysporum* ranged between 60–97 nm. **Sarkar *et al*. (2011)** showed that the diameter of Se NPs by *A. alternata* was measured in the range of 30–150 nm. In addition, the study carried out by **Abbas & Abou Baker (2020)** on bio-Se NPs by *F. semitectum*, showed that their diameter ranged from 32.80 nm to 103.82 nm. Furthermore, the average particle size of the biosynthesized Se NPs produced by *F. oxysporum* was 42nm (**Islam *et al*., 2022**).

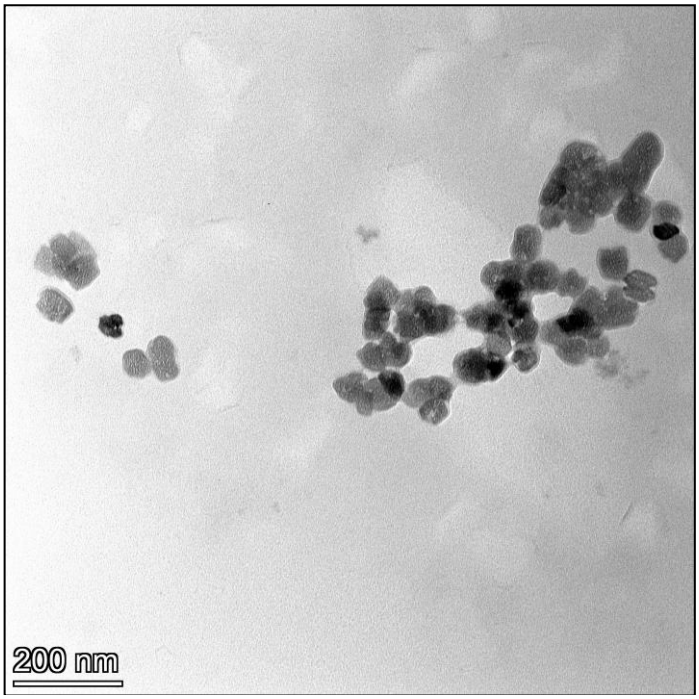

Figure 5 Transmission electron microscope of AMP/CS/SeNC. Bars scale = 200 nm.

Antibacterial activity and MIC

The antibacterial activity of Se NPs, AMP, and CS was individually shown and compared with the antibacterial action of AMP/CS/SeNC in Figure 6 and Table 1. The results showed that AMP/CS/SeNC had a bigger effect on the multidrug-resistant *E. coli* (AC: PP797596) bacteria than Se NPs and AMP by itself. MIC values of AMP/CS/SeNC were found to be 30 µg/ml compared to CS; 190 µg/ml, AMP; 200 µg/ml, and Se NPs; 160 µg/ml (Figure 7). All tested compounds displayed a dose-dependent behavior of antibacterial potential.

Many investigations confirmed that all the tested Gram-positive strains were sensitive to Se NPs; however, the Gram-negative bacteria showed significant resistance to Se NPs within a wide range of concentrations, according to **Tran *et al*. (2016)** and **Souza *et al*. (2022)**. Furthermore, **Guisbiers *et al*. (2016)** reported that 50 ppm of the Se NPs sample exhibited the highest inhibition rate (46% of *E. coli* growth) after 24 h. On the other hand, **Nguyen *et al*. (2017)** showed that the number of treated cells of *E. coli* O157:H7 after 10 h of incubation reduced marginally (less than 1 log CFU/ml) in comparison to the control, with a concentration of Se NPs (50 mg/ml). This suggests that Se NPs had a negligible effect on *E. coli* O157:H7. Additionally, **Vahdati & Tohidi Moghadam, (2020)** recorded that the highest inhibition (41%) was observed at 660 µg.ml^{-1} of Se NPs against *E. coli*.

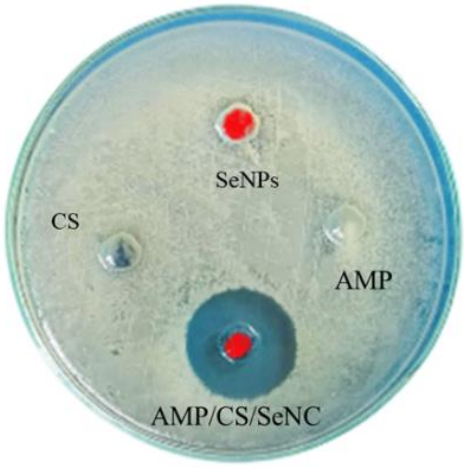

Figure 6 The agar well diffusion method of AMP/CS/SeNC compared to CS, AMP, and Se NPs at the concentration of 150 µg/ml against the tested *E. coli* strain.

Table 1 Antibacterial activity of different compounds against the tested E. coli strain

Compounds	Concentration (µg/ml)	Inhibition zone in mm (mean ± SD)
CS	50	-ve
	100	-ve
	150	7 ± 0.14
Se NPs	50	-ve
	100	-ve
	150	8 ± 0.19
AMP/CS/SeNC	50	21 ± 0.06
	100	24 ± 0.03
	150	27 ± 0
Ampicillin	50	-ve
	100	-ve
	150	6 ± 0.21

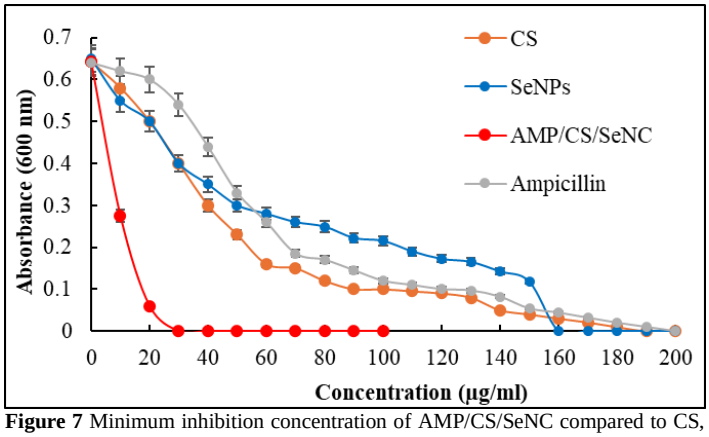

Figure 7 Minimum inhibition concentration of AMP/CS/SeNC compared to CS, AMP, and against the tested *E. coli* strain.

Effect of AMP/CS/SeNC on *E. coli* ultrastructure

Morphological characteristics of the ultrastructure of *E. coli* were altered due to the effects on cells subjected to AMP/CS/SeNC (Figure 8). Untreated cells appeared as intact rods with incorporated cell walls. On the contrary, the cell walls of the AMP/CS/SeNC-treated cells were recognized to be wrinkled and crushed, and a clear visible separation between the plasma membrane and cell wall was also observed. In addition to the presence of completely decomposed cells as a result of the AMP/CS/SeNC effect.

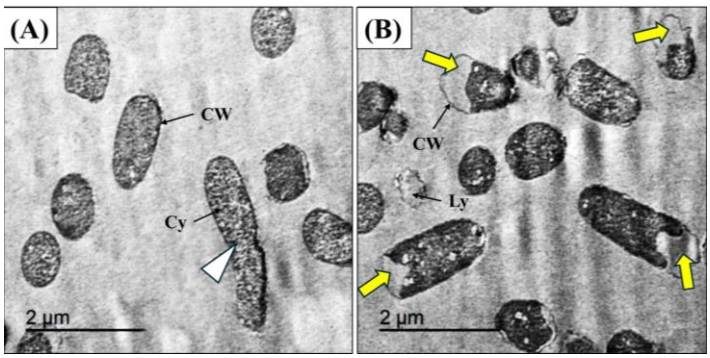

Figure 8 TEM micrograph of AMP/CS/SeNC-treated *E. coli* cells; (B) compared to control cells; (A). CW; cell wall, Cy; intact and homogenous cytoplasm, Ly; complete lysed cells. Note, the binary fission of bacterial cells at the white arrowhead (control cells) and the separation between cell wall and plasma membrane at the yellow arrows (treated cells).

The potential mechanism for inhibiting *E. coli* bacteria is that Se NPs bind via chemisorption **(Kieliszek *et al*., 2015)** along with penetrating the outer membrane containing lipopolysaccharides, which are covalently bonded to the cell's peptidoglycan by Braun's lipoprotein **(Silhavy *et al*., 2010; Mohamed & El-Zahed, 2024)**. Three enzymes, preprolipoprotein diacylglyceryl transferase, prolipoprotein signal peptidase, and apolipoprotein N-acyltransferase, contribute to the essential lipoprotein biosynthesis pathway in *E. coli* bacteria. It has been demonstrated that these enzymes are critical to *E. coli* survival. Therefore, by altering the function of these enzymatic transporters, Se NPs inhibit *E. coli*. As a result, Se enters far more effectively by chemisorption, a process that involves the diacyl and triacyl forms of lipoproteins **(Nakayama *et al*., 2012)**. Thus, the cell wall and the polysaccharide components that make up the wall act as a barrier that restricts the amount of Se NPs that may enter the inside of the cell.

The low-cost AMP/CS/SeNC nanocomposite's enhanced antibacterial efficiency, lowered dosage needs, and effectiveness against multidrug-resistant bacteria make it more cost-effective than traditional therapies. Conventional ampicillin is less cost-effective in the long term because of its greater dosage requirements, chance of treatment failure, and requirement for fighting growing resistance. The current study provides the AMP/CS/SeNC nanocomplex as a cost-effective and sustainable therapy choice which could provide higher value in the form of improved health outcomes, less hospitalization expenditures, and less environmental impact than other chemical antibacterial agents. However, further study is required to determine the ideal Se dosage to increase ampicillin's effectiveness while lowering the risk of Se toxicity. Bioavailability and clinical risks should be examined between various statements (oral, intravenous). It's critical to evaluate how effectively Se and ampicillin work together to combat a range of multidrug-resistant bacteria.

CONCLUSIONS

In summary, the current study demonstrated the potency of *F. fujikuroi* PP794203 for the first time reported to mycosynthesize of Se NPs. The synthesized nanocomposite (AMP/CS/SeNC) exhibited antibacterial potential against the multidrug-resistant *E. coli* PP797596. The ultrastructure of the treated *E. coli* cells confirmed the high antibacterial potential of the AMP/CS/SeNC. Finally, the biosynthesized AMP/CS/SeNC exhibited generally acceptable effectiveness and stability, thus it is recommended to be used as an interesting bacterial growth inhibitor. Furthermore, the toxicity, antimicrobial activity, and stability of AMP/CS/SeNC needs to be studied *in vivo* with an animal model.

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