Potent antibacterial nanocomposites from okra mucilage/chitosan/silver nanoparticles for multidrug-resistant *Salmonella* Typhimurium eradication

Abstract: The polymeric nanocomposites (NCs), constructed from okra (*Abelmoschus esculentus*) fruits mucilage (OM), silver nanoparticles (AgNPs), and chitosan (Ch), were fabricated as potential candidates to overcome drug-resistant *Salmonella* Typhimurium bacteria. AgNPs were directly mediated by OM, with 4.2 nm mean diameters. The composed NCs from Ch/OM/AgNPs were innovatively synthesized and the various ratios of Ch:OM/AgNPs affected the NCs particles’ size and charges. The infrared analysis of employed materials/NCs validated their interactions and conjugations. The antibacterial assays of NCs against different resistant *S.* Typhimurium strains indicated the efficiency of polymeric NCs to inhibit bacteria with significant superiority over standard antibiotics. The NCs that contained equal ratios from Ch and OM/AgNPs were the best formulation (mean diameter, 47.19 nm and surface charge, +16.9 mV) to exhibit the strongest actions toward *S.* Typhimurium. The NCs caused severe deformation, destruction, and lysis in exposed bacteria, as traced with scanning microscopy. The biosynthesis of AgNPs using OM and their nanoconjugation with Ch provided eventual natural biopolymers NCs with enhanced expected bio-safety and efficiency against drug-resistant *S.* Typhimurium strains, which supports their potential applications as disinfectant, sterilizing, and curative antibacterial agents.

Keywords: antimicrobial, bactericidal, biopolymers, biosynthesis, nanoconjugation

1 Introduction

Biopolymers are beneficial polysaccharides attained from natural sources (e.g., plants, microbes, algae, and marine sources); they could be supremely applied in numerous therapeutic/pharmaceutical products due to their renewability, biocompatibility, and availability [1]. The nanoforms of biopolymers could provide further bioactivities and potentialities to assist the functionalities of other biomolecules [2]. From these biopolymers, plant mucilages and marine polymers were proposed to investigate their potential bioactivities.

Mucilages are mainly plant polysaccharides that are intracellularly formed, with varied molecular structures [3]; the mucilage polysaccharides could demonstrate some vital bioactivities (e.g., anti-inflammatory, immune-modulatory). Mucilages are also perfect candidates for micro-/nano-encapsulation of other bioactive molecules, phytochemicals, and probiotics; their edibility, solubility, stability, and biodegradability advocated their practical health applications [4]. Recently, many plant mucilages were applied to develop nanomaterials, including nanocapsules, nanofibers, and nanocomposites (NCs) or biosynthesis and encapsulation of numerous bioactive molecules and nanometals as an innovative protocol [5–8].
Okra fruits/pods (Abelmoschus esculentus) contribute to public diet/local dishes of many countries because of vital nutritional constituents (e.g., protein, fiber, minerals); okra is frequently utilized for thickening and viscous constancy in numerous soups/broth. Okra pods have considerable amounts of calcium, iron, and zinc and contain low amounts of antinutrients with high mineral bioavailability [9]. Okra mucilage (OM) comprises randomly coiled polysaccharides that contain galactose, galacturonic acid, and rhamnose; the repeated units in the OM structure are (1-4)-galacturonic acid and (1-2)-rhamnose with some disaccharide side chains [1,6]. OM, like other biopolymers, has many advantages compared to synthetic polymers; they are safe, nonirritant, biocompatible, eco-friendly, biodegradable, and chemically inert [10]. OM is daily consumed in regular diets and therefore guaranteed for biosafety and compatibility without toxicological studies [11]. The high viscous and slimy properties of OM could be advantageous as an encapsulating polymer for formulating sustained-release medications [1,11]. OM was investigated and demonstrated as a functional nutritional ingredient with numerous bioassays; OM possessed remarkable antimicrobial, antioxidant, hypoglycemic, antitumor, anti-ulcer, anti-toxication, and cholesterol-lowering capacities [10–15].

From the most promising marine biopolymers, chitosan (Ch), which is a positively charged active biopolymer derived after chitin deacetylation, comprises linear units of N-acetyl-D-glucosamine and dissolves in low pH solutions due to the reactivity of its basic amino groups with acidic radicals. The cationic attributes of Ch provide remarkable functionalities such as antimicrobial capabilities (through interacting/attaching anionic microbial membranes, DNA, RNA, and enzymes), encapsulation efficiency (via upholding of further negatively charged biomolecules and nanomaterials), biosorption (by adoption of numerous toxic and hazardous molecules), and anticancer potentiality (e.g. interactions with cancer cells and their interior organelles) [16–18].

Composited organic/inorganic materials from diverse types that have one phase (at least) in the “nano” scale are called NCs [19]; NCs have advantageous characteristics over their parent materials with prominent bioactivities and functionalities [19,20]. Ch (in its bulk or nano form) has an extraordinary ability to evolve in bioactive NC constructions with other biopolymers, phytocompounds, and nanometals [8,21–23].

The metallic nanoparticles (i.e., nanometals) were employed in numerous biomedical and pharmaceutical applications, and the antimicrobial effectiveness of nanometals was documented, basically due to cytotoxicity toward microbial pathogens and interactions with their membranes/interior pathways [24–27]. Mostly, silver nanoparticles (AgNPs) were extensively documented for their antimicrobial actions [24,25], involving Ag⁺ ions release, ROS (reactive oxygen species) generation in the inner/outer microbial membranes, interference with the cellular membrane, ribosome-mitochondrial disruption, and nucleic acid interruptions. The biological synthesis of AgNPs is much more advantageous compared to physical synthesis and chemical-based approaches; biosynthesis is biosafe, environmentally friendly, energy- and cost-saving, which generates homogenous and bioeffective NPs [25–27].

The encapsulation/conjugation of AgNPs within biopolymer and phytocompound coats can impressively minimize their probable toxicities to human cells/tissues while promisingly preserving or slightly increasing the bioactivities toward microbial pathogens and invading cells [28–31].

The global foodborne infections by Salmonella spp. exceeded 115 million humans annually, resulting from diverse serovars and strains [32]. Among them, S. Typhimurium, “i.e. Salmonella enterica serovar Typhimurium,” caused most the non-typhoidal salmonellosis in humans worldwide. The resistances of S. Typhimurium to multiple antibiotics were reported in different countries, antibiotics, and isolates [33].

Accordingly, the aims of the present work were the extraction of OMs and their innovative usage for AgNP biosynthesis, the construction of bioactive NCs from Ch, OM/AgNPs, and to employ them as antibacterial candidates to suppress S. Typhimurium-resistant strains.

2 Materials and methods

2.1 Materials and reagents

The organically cultivated okra (Abelmoschus esculentus) fruits, with 24–28 mm length, were obtained from the ARC “Agricultural Research Center, Giza, Egypt.” All chemicals, microbiological media, reagents, and dyes used in the current study (e.g., sodium hydroxide [NaOH]; ethanol [96%]; silver nitrate [AgNO₃]; chitosan [≥80% deacetylation degree; ∼100 kDa molecular weight; CAS 9012-76-4]; sodium tripolyphosphate; acetic acid glacial; potassium bromide [KBr]; and p-iodonitrotetrazolium violet dye) were purchased from Sigma-Aldrich Co. (St. Louis, MO), unless other source is specified.

2.2 Extraction of OM

OM was extracted from A. esculentus fruits, as adapted from Freitas et al. [34]. First, the okra fruits were manually...
sliced to obtain a 2–3 mm height using a stainless steel cutter, and the resulting pieces were immersed in DIW (deionized water) at 1:30 w/v ratio at room temperature (RT, 25 ± 2°C). Using 1 M NaOH, the pH value was adjusted to 10.1 ± 0.2. Afterward, this mixture was thoroughly stirred for 45 min at RT (~25 ± 2°C). The OM was separated from okra fruit residues by pressing through a double sterile cloth filter. For OM precipitation, an equal volume of ethanol (96%) was mixed with the former extract, and their mixture was statically kept at a cold (4 ± 1°C) temperature for 125 min. The precipitated OM was detached via centrifugation (4,650 × g) and freeze-dried.

2.3 Preparation of OM/AgNPs

The procedure for AgNP reduction/mediation using OM was modified from a recent protocol [35]. First, fresh solutions of AgNO₃ (10 mM) and OM (0.1%, w/v) were separately prepared in DIW at RT, using stirring at 230 × g for 60 min. The solutions were passed through a syringe (0.45 µm) filter, and 25 mL of OM solutions were dropped in equal amounts of AgNO₃ solution (while stirring at 580 × g) for 95 min. The color change of the mixture solution into blackish brown could be visually seen as an indicator of OM/AgNPs formation. The formed NPs were harvested from the solution through 35 min centrifugation at 9,800 × g (2-16 KL, Sigma; Osterode am Harz, Germany), washing with DIW, re-centrifugation, and freeze-drying. To attain plain AgNPs, the DIW washing and re-centrifugation processes were repeated five times to eliminate most of the attached OM.

2.4 Construction of Cht/OM/AgNPs NCs

The assembly of the NCs from Ch/OM/AgNPs followed these steps [8]: solutions of 0.1% (w/v) were made from OM/AgNPs (in DIW) and Ch (in 1.5%, v/v aqueous acetic acid solution); the solutions were individually sonicated for 14 min. About 200 mg of TPP (Na-tripolyphosphate) was dissolved in 20 mL of OM/AgNP solution and re-sonicated. Then, the OM/AgNPs-TTP solution was gently dropped (~ 20 mL·h⁻¹) into Ch solution under speedy stirring (735 × g) for NC formation:

- F₁ (2 OM/AgNPs: 1 Ch)
- F₂ (1 OM/AgNPs: 1 Ch)
- F₃ (1 OM/AgNPs: 2 Ch).

Stirring was continued for a further 40 min, and then the formed NCs were harvested via centrifugation (10,720 × g), DIW washing, recentrifugation, and freeze-drying. For plain Ch formation, the solvated TPP of step 4 was directly dropped without the Om/AgNP amendment.

2.5 Nanomaterial/NC characterization

2.5.1 Fourier transform infrared (FTIR) spectroscopy analysis

FTIR spectroscopy was utilized for analyzing the functional groups/biochemical bonds in the materials/NCs employed in the investigation and their interactions, including Ch, OM, OM/AgNPs, and Ch/OM/AgNPs. FTIR (JASCO, FT-IR-360, Japan) spectra were appraised after mixing materials/NC powders with KBr; their IR spectra (transmission mode) were measured in the wavenumber range of 4,000–450 cm⁻¹.

2.5.2 Nanomaterial size and charge

The constructed nanomaterials/NCs were dissolved in DIW and sonicated at 43 W for 15 min. Then, the distribution characteristics of particle sizes (Ps), zeta (ζ) potential, and charges were estimated using a Malvern™ Zetasizer (Worcestershire, UK) at RT, employing the dynamic light scattering (DLS).

2.5.3 Scanning (SEM) and transmission (TEM) electron microscopy

SEM (JEOL IT100, Tokyo, Japan) and TEM (Leica; Leo-0430; Cambridge, UK) imaging elucidated the nanomaterial/NC morphology, Ps, and distributions. DIW suspensions of nanomaterials/NCs (OM/AgNPs, F₁, F₂, and F₃) were sonicated for inspection. The OM-mediated AgNPs were inspected via TEM after dropping onto copper grids, vacuum-dehydrating for 32 min, and subjecting to TEM imaging at 20 kV. The NC solutions were SEM screened after mounting onto carbon discs (self-adhesive), coated with palladium/gold (the coater: E5100 II, Polaron Inc., PA), and inspected at 10–15 kV operating acceleration.

2.6 Anti-Salmonella activity

2.6.1 Bacterial strains

Different strains of Salmonella Typhimurium “Salmonella enterica ssp. enterica serovar Typhimurium” were screened
in challenging assays; they included the standard strain ATCC-700408 (multi-drug-resistant) and the isolates M and H (isolated from minced beef and worker’s hands, respectively). The *S. Typhimurium* isolates were principally identified based on Gram stain, catalase/oxidase reaction, morphology, and endospore formation. Standardized protocols (ISO 6579-1:2017 and ISO/TR 6579-3:2014) were followed to identify the *Salmonella* strains [36,37]. The identification was further continued with (an API automated system) for the analytical profile index “BioMérieux Vitek-II System, France” according to the manufacturer’s instructions and further confirmed using 16S rRNA analysis [38]. The used isolates exhibited multiple drug resistances to ampicillin, tetracycline, streptomycin, and sulfonamide. Nutrient agar/broth (NA and NB; Difco Lab., Detroit, MI, USA) was used for bacterial activation and propagation and challenged aero-bically at 37 ± 1°C.

### 2.6.2 Qualitative inhibition zone (IZ) antibacterial assay

The experiments were mostly conducted without direct light sources (e.g., in the dark) to eliminate the probable effects of light on NP antimicrobial activity. The *S. Typhimurium* strain cells spread on NA media were individually challenged using paper assay discs (6 mm diameter) that carried 25 µL of nanomaterials/NC solutions (with 10 mg·mL⁻¹ concentration), and discs were positioned onto inoculant surfaces. These discs were loaded with Ch, OM/AgNPs, F1, F2, and F3 NCs, and the challenged plates were incubated for 28–36 h until the IZ development. The mean diameters of IZs were measured and computed.

### 2.6.3 Quantitative minimum inhibitory concentration (MIC)

The MICs of Ch, OM/AgNPs, F1, F2, and F3 NCs against challenged *S. Typhimurium* strains were gauged using the “micro-dilution” protocol [39]. The grown Salmonella cultures in NB (3 x 10⁶ cells·mL⁻¹) were challenged by successively amended concentrations from nanomaterials/NCs (within 1–100 µg·mL⁻¹ range), using (96-well) microplates. Then, a chromogenic indicator (p-iodonitrotetrazolium violet; 4% w/v) was added to the challenged microplate wells after 20 ± 2 h of incubation, which provides red formazan color with cells’ viability. For bactericidal action confirmation, 100 µL from colorless wells that were suspected to have MICs were plated onto fresh plain NA plates to detect potential growth. MICs indicate the least nanometal concentrations, which inhibited exact bacterial development in microplates and subsequent NA plates.

### 2.6.4 SEM observation of NC-challenged bacteria

After exposure to Ch/OM/AgNPs (F2 formulation), at relevant MIC values against *S. Typhimurium* ATCC-700408, cells were incubated for 3, 6 and 9 h, and the apparent morphological variations after treatments were photographed by SEM. The logarithmic-grown *S. Typhimurium* cells were amended by the NC in NB. Then, cells were harvested via centrifugation (4,850 × g), washed with saline buffer, dehydrated with ethanol on SEM stubs, gold/palladium coated, and screened for structural distortion/deformation compared to control cells.

### 2.7 Statistical analysis

SPSS v17.0 package software (SPSS Inc., Chicago, IL, USA) was used for statistical data analysis. Standard deviations and averages after triplicate measurements were calculated; the significances between them were computed using ANOVA (one-way) and *t*-tests, with *p* ≤ 5%.

### 3 Results and discussion

The aims of the current research were the fabrication of OM-mediated AgNPs and their conjugation with Ch to generate bioactive antimicrobial NCs against *S. Typhimurium*. The nanomaterials/NCs were synthesized using facile protocols and characterized to assess their attributes and antibacterial bioactivities.

#### 3.1 FTIR assessment

FTIR analysis of composite materials can highlight their major biochemical groups/bonds and reveal the effects of interactions between the components (Figure 1). The patterns of interacted materials (e.g., OM, OM/AgNPs, Ch, OM/Ch, and Ch/OM/AgNPs) illustrated their basic biochemical groups and their changes after nano-compositing.

For OM, the existence of many peaks around 3,420 cm⁻¹ could represent the vibrated stretches of N–H and O–H bonds of carboxylic acid; the –OH presence in the pattern employs the hydrophilic appeal of that polymer. The 1,738.5 cm⁻¹ peak is indicative of C=O stretch (mainly in alacturonic acid), whereas the region of 1,230–868 cm⁻¹ could represent a carbohydrate fingerprint [40,41]. The IR spectrum at
1,636.8 cm\(^{-1}\) is attributed to the ionized carboxyl, whereas the 2,921.6 cm\(^{-1}\) band is attributed to the \(-\text{CH}_2\) groups in cellulose/hemicelluloses and the 1,738.5 cm\(^{-1}\) is attributed to the ester carbonyl of OM \([5,42,43]\). Additionally, the so-called “fingerprint region,” which is attributed to polysaccharides, could be detected between 1,400 and 1,000 cm\(^{-1}\) \([44]\), and these representative polysaccharides’ bands were documented also within 1,200–950 cm\(^{-1}\) \([34]\). According to these fingerprints, the 1,254.4 cm\(^{-1}\) band was assigned to C–O stretching, whereas the 1,052.1 cm\(^{-1}\) referred to C–O–C group stretching in complex polysaccharides. Furthermore, the 1,628.2 cm\(^{-1}\) band could be assigned to amide I and is located in a highly sensitive region of proteins’ secondary structures and attributed to stretched C–O vibrations of peptides’ linkages \([45]\). This remark suggested that, besides polysaccharides, the OM could also comprise some proteinaceous materials, which is expected as the biopolymer extraction did not involve the deproteinization step \([44]\). The band at 3,273.4 cm\(^{-1}\) signified the intra- and inter-molecular H-bonding within associated hydroxyl groups to the carbohydrate structure \([46]\).

The reduction and conjugation of AgNPs with OM resulted in notable alterations in their IR spectra (Figure 1, OM/Ag) compared to the plain OM spectrum. The spectra changes included the disappearance of several bands (e.g., at 621.3 cm\(^{-1}\), within the 1,288.4–1,446.3 cm\(^{-1}\) range, at 1,623.8, 1,686.5, and 3,620.3 cm\(^{-1}\)), which specify that the disappeared groups interacted with AgNP ions and broke their bonds through biosynthesis/reduction. The changes also included the emergence/sharpening of numerous peaks in the OM/AgNP spectra (e.g., at 491.6 and 762.4 cm\(^{-1}\), within the 894.5–1,119.6 cm\(^{-1}\) range, 1,788.9–2,863.1 cm\(^{-1}\) range and at 3,404.6 cm\(^{-1}\)), which markedly indicate the development of novel bonds amid OM biomolecules with AgNPs \([8,35,47]\).

The IR spectrum of Ch (Figure 1, Ch) could assign the key bonds/groups that specify standard chitosan \([20,48]\). The key characteristic indicators in the plain Ch spectrum were perceived around 3,425 cm\(^{-1}\) (stretching vibration of N–H and O–H groups), 2,924.2 cm\(^{-1}\) (stretching vibration of C–H aliphatic groups), 1,657.5 cm\(^{-1}\) (stretched vibration of amide II NH bonds), 1,116.4 cm\(^{-1}\) (–OH vibrational stretching in the C3 carbon), and 1,033.8 cm\(^{-1}\) (–OH vibrational stretching in the C6 carbon) \([16,49,50]\). Consistently, in the Ch spectrum, the wide band around 3,420 cm\(^{-1}\) is the key band for interaction with cross-linker agents and other biomolecules in the Ch-based NCs \([21,48]\).

The OM/Ch/AgNPs show the NC’s main bonds/groups (Figure 1), which reflect the consequences of component interactions. In the NC spectrum, several bands appeared to be derived from Ch (indicated with red zones), and others were derived from OM/AgNPs (indicated with green zones), which could affirm physical and electrostatic conjugation with combined components, mostly because of dissimilar charges of these components \([8,51]\).

### 3.2 Optical assessments of OM-mediated AgNPs

The bioreduction/transformation of AgNO\(_3\) to AgNPs, via OM mediation/stabilization, could be perceived visually (Figure 2). The color of the OM and AgNO\(_3\) solution mixture changed from faint yellow to blackish brown within 40 min of the bioreduction process (Figure 2a).
The spectroscopic (UV-vis) analysis of OM-mediated AgNPs authenticated their reduction/transformation into nanoform (Figure 2b), which was verified by the lambda-max (420 nm) of the reduction solution color. The TEM images of the OM-synthesized AgNPs could verify the formation and dispersion of NPs, with an average diameter of 4.32 nm (Figure 2(T)).

Optical characterization shows the increased potentiality of OM to generate AgNPs, which is basically attributed to the surface plasmon resonance (SPR) of biosynthesized AgNPs that was reported to have a λ max of around 420 nm [27,52,53]. The UV sharp single peak and the dark blackish color of the biosynthesis matrix confirmed the efficacy of OM in reducing, stabilizing, and capturing AgNPs [23,54].

Additionally, the elemental analysis via energy dispersive X-ray (EDX) that coupled with TEM showed the predominance of Ag, C, and O elements in the OM-biosynthesized AgNPs (supplementary materials; Figure S1).

3.3 Structural attributes

The physiognomies of nanomaterial/NC structures (e.g., OM/AgNPs and the constructed NCs from Ch:OM/AgNPs) were screened through DLS examination (Table 1) and electron microscopy (Figure 3). The parent components (e.g., Ch and OM/AgNPs) carried contrasted charges (+37.5 and −26.3 mV, respectively), and their mean particles greatly differed (>1,000 and 4.21 nm, respectively), as shown in Table 1 and Figure 2(T).

The mean sizes and charges of constructed NCs (F1, F2, and F3) indicated the effect of Ch:OM/AgNP mixing ratios on the generated NC particles (Table 1 and Figure 3). The SEM images of NC formulations illustrated consistent sizes with the measured sizes by DLS analysis; the least average NC size was achieved in F2 (47.19 nm), followed by F3 and then F1 composites (54.58 and 78.65 nm, respectively). The F1 composites had negative surface charges (−18.6 mV), and both F2 and F3 formulations carried positive charges (+16.9 and +27.3 mV, respectively). The microscopic images emphasized the well-distributed and miniature NC particles, especially in the F2 construction (Figure 3b).

The presented innovative protocol for the production of Ch/OM/AgNP composites (F1, F2, and F3) succeeded in generating diminished NC particles, as verified by

Table 1: Physiognomies of screened materials/NCs

<table>
<thead>
<tr>
<th>Nanomaterials</th>
<th>Size range (nm)</th>
<th>Mean size (nm)</th>
<th>Charge (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OM/AgNPs</td>
<td>1.91–16.72</td>
<td>4.21</td>
<td>−26.3</td>
</tr>
<tr>
<td>Ch</td>
<td>&gt;1,000</td>
<td>&gt;1,000</td>
<td>+37.5</td>
</tr>
<tr>
<td>F1 (2 OM/AgNPs: 1 Ch)</td>
<td>33.26–203.18</td>
<td>78.65</td>
<td>−18.6</td>
</tr>
<tr>
<td>F2 (1 OM/AgNPs: 1 Ch)</td>
<td>20.12–128.77</td>
<td>47.19</td>
<td>+16.9</td>
</tr>
<tr>
<td>F3 (1 OM/AgNPs: 2 Ch)</td>
<td>28.80–195.53</td>
<td>54.58</td>
<td>+27.3</td>
</tr>
</tbody>
</table>
preceding examinations. This nanoconjugation was recently introduced using other plant mucilage (garden cress seeds) and nanometals (i.e., selenium NPs) [8], which validated this protocol for effectual synthesis of biopolymer NCs. The key suggested factor for generating such biopolymer NCs is the apparent opposite charges carried onto parent components’ surfaces (e.g., positive in Ch and negative in interacting materials). The interaction and formation of NCs among oppositely charged biopolymers were stated earlier; they involved Ch as the positively charged polymer in addition to other negatively charged types (e.g., alginate, fucoidan, carrageenan, ulvan, gums, nanometals) [8,22,26,55]. The resultant NCs from these conjugations normally have higher stability, bioactivity, and bio-functionality [8,26,56].

Herein, the anionic OM/AgNP complex could interact with the cationic Ch to develop an innovative polyelectrolyte complex (PLC); the net surface charge of such PLC mainly depends on the involved ratios from component electrolytes [56]. The value of NC charges (Zeta potentiality) can significantly affect their stability/dispersion inside aqueous suspensions through repulsed electrostatics between particles, which is of influential importance for NC bioactivities [57].

3.4 Anti-Salmonella Typhimurium activities

The antibacterial potentialities of biomaterials/NCs (OM/AgNPs, Ch, F1, F2, and F3 formulations) against S. Typhimurium isolates were proven using qualitative/quantitative assays (Table 2). The fabricated NCs (F1, F3, and F3) exhibited more forceful antibacterial actions compared to their parent components (Ch and OM/AgNPs). This was manifested by the wider IZ diameters and lesser MICs. Regarding these measurements, the most

Table 2: Anti-Salmonella Typhimurium activities of biosynthesized silver nanoparticles with okra mucilage (OM/AgNPs), chitosan (Ch), and their composites against different isolates from drug-resistant strains*

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>S. Typhimurium ATCC</th>
<th>Salmonella Typhimurium strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IZ (mm)</td>
<td>MIC (µg·mL⁻¹)</td>
</tr>
<tr>
<td>OM/AgNPs</td>
<td>17.2 ± 0.9a</td>
<td>35.0</td>
</tr>
<tr>
<td>Ch</td>
<td>11.3 ± 0.6b</td>
<td>&gt;100.0</td>
</tr>
<tr>
<td>F1 (2 OM/AgNPs: 1 Ch)</td>
<td>23.8 ± 1.5c</td>
<td>10.0</td>
</tr>
<tr>
<td>F2 (1 OM/AgNPs: 1 Ch)</td>
<td>27.3 ± 2.3d</td>
<td>7.5</td>
</tr>
<tr>
<td>F3 (1 OM/AgNPs: 2 Ch)</td>
<td>19.8 ± 1.2e</td>
<td>12.5</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>17.6 ± 0.8f</td>
<td>15.0</td>
</tr>
</tbody>
</table>

*Dissimilar letters within a column (superscripts) designate significance of differences (p ≤ 0.05).
effectual NCs were F2 (1 OM/AgNPs: 1 Ch), which exhibited the highest efficacy toward the entire challenged strains. F2 could significantly exceed the action of chloramphenicol. The subsequent bioactive NCs were F1 and F3, respectively.

The bacterial strain responses varied toward examined antibacterial NCs; S. Typhimurium M was the most susceptible strain, whereas S. Typhimurium H exhibited the uppermost resistance among challenged strains (Table 2). The variation in bacterial resistance pattern to NCs validated that they belonged to different strains.

The key antibacterial agents in OM were illustrated to include many antinutrient components (e.g., tannins, phytic acid, essential oils, and flavonoids), which affect bacterial development [6]. Additionally, some lipid components of OM (mainly stearic and palmitic acids) were suggested for the antimicrobial potentiality of OM-based films [7,58]. Some investigations appointed that OM-based composites with other biopolymers (e.g., alginate, starch, carboxymethyl cellulose) have enhanced ability to carry/encapsulate bioactive molecules (e.g., zinc oxide, oxcarbazepine) and improve their functionalities (e.g., as antimicrobial agents) [5–7]. Current results show agreements with these investigations as the OM-based NCs with Ch could strengthen the AgNP biocidal activity toward S. Typhimurium strains. Numerous investigations highlighted the powerful action of AgNPs to control drug-resistant microorganisms, particularly with AgNPs embedding within the nanopolymer matrix (e.g., Ch, cellulose, alginate, and their composites) [25]. The main AgNPs’ antibacterial actions are attributed to the ions’ ability to bind/interact with thiol groups (R-SH) in bacterial membrane proteins to obstruct respiration and biofunctions of cell walls [24]. As the Ch cationic nature facilitates its attachment/attraction with the negative bacterial membranes, Ch was suggested as an ideal nanocarrier to deliver antimicrobial agents (e.g., OM/AgNPs here) to the surface or inside the cells to perform their destructive actions [17].

The associations between NC surface charges and their biocidal actions (e.g., bactericidal and anticancerous) were reported [8,47,59–61]; F2 (positively charged NCs) had the strongest antibacterial activity here, which involves their ability to attach to the negatively charged bacterial membranes.

3.5 SEM screening of morphological deformation of NC-exposed bacteria

The results of exposure to the Ch/OM/AgNP composite on the morphology, structure, and manifestation of S. Typhimurium ATCC-700408 are presented in Figure 4. For an imaginable explanation of Ch/OM/AgNPs (formulation F2) antibacterial actions, SEM visualizations were screened against the standard S. Typhimurium drug-resistant strain. The 0-time exposed cells exhibited ordinary, healthy, and uniform structures; no distortion/deformation signs were observed (Figure 4(T0)). After exposure to Ch/OM/AgNPs for 3 h (Figure 4(T3)), remarkable deformation/distortion signs were initiated on bacterial membranes, with observable attached NCs to cell surfaces. The signs of cells’ deformations/destinations were observable after 6 h exposure to Ch/OM/AgNPs (Figure 4(T6)); the NC particles covered most of the treated cells.

Figure 4: Scanning images of treated Salmonella Typhimurium cells with the NC from biosynthesized silver nanoparticles with okra mucilage and chitosan, illustrating the control (T0) and after treatment for 3, 6, and 9 h.
Many irregular/inconstant cell shapes appeared in this stage, with observable cells residues after their lysis. After 9 h exposure to Ch/OM/AgNPs, most treated S. Typhimurium cells were lysed/decomposed; their interior exudates and membrane residues that conjugated the NC particles were most observable (Figure 4(T9)). The matched SEM observations were recorded formerly after the exposure of varied bacterial types to Ch-based NCs in conjunction with other biopolymers, nanomaterials, and phytochemicals [8,23,26]. The synergism of biocidal activities after nanoconjugation of Ch with other biomolecules (e.g., the F1, F2, and F3 formulations here) in comparison with their parent constituents (e.g., Ch, OM, and AgNPs) was earlier attributed to Ch capabilities (depending on surface positivity) to develop NCs through encapsulation of further molecules from bioactive nanomaterials; these NC bioactivities involve the attachment into negative bacterial cells/membranes, interruption of their permeability, and obstructing bacterial biosystems [23,27,48].

The Ch biocidal action was shown from captured SEM images that elucidated the adherence of Ch/OM/AgNPs onto bacterial cells and assumingly their penetration inside the cells to destroy their vital biosystems [21,23,26,55,62].

SEM images formerly showed that Ch-based bioactive NCs could possess multiple antibacterial mechanisms via attaching to negatively charged cell walls/membranes, suppressing the membrane synthesis, affecting membranes’ permeability, penetrating the innermost cells, leaking vital cellular components, suppressing the metabolic pathways/functions, and inducing the apoptosis-like death in bacterial cells [23,55,62].

Besides the surface positivity of (F2) NC that could facilitate its attachment onto negative bacterial walls/membranes and/or vital components, the NC content of biosynthesized nanomaterials (e.g., AgNPs) was validated to possess the powerful antibacterial actions that principally depend on NP cytotoxicity toward targeted cells via inactivation/interaction with their physiological pathways [29,63,64]. The main limitation of the current protocol was the potential biotoxicity of AgNPs, which could be overcome by the biosynthesis of nanomaterials using organic matter and their nanoconjugation with the Ch biopolymer [65–67]. Nearly all the materials/components used were derived from natural sources, which warrant the non-toxicity, biocompatibility, and sustainability of the process and minimize the potential biotoxicity of AgNPs [66,68,69].

4 Conclusions

This research targeted the generation of bioactive antimicrobial NCs against S. Typhimurium through the fabrication of OM-mediated AgNPs and their conjugation with Ch. The nanomaterials/NCs were successfully synthesized using facile protocols and characterized to assess their attributes and antibacterial bioactivities. OM could mediate AgNP reduction/stability with a mean Ps of 4.21 nm. The biopolymers formulated NCs of Ch/OM/AgNPs, which were extraordinarily validated as effective anti-Salmonella Typhimurium nano-medication. The formulation F2 (contained equal ratios of OM/AgNPs and Ch), with a mean size of 47.19 nm and a surface charge of +16.9 mV, were the most effectual antibacterial NCs against S. Typhimurium strains and could significantly exceed standard antibiotic actions. The biosynthesis of AgNPs using OM and their nanoconjugation with Ch provided effectual natural biopolymer NCs with enhanced expected biosafety and efficiency against drug-resistant S. Typhimurium strains, which supports their potential applications as disinfectant, sterilizing, and curative antibacterial agents.

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