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Antimicrobial: A Comparative Study of Silver Nanoparticles and Chitosan Hydrogel Effect on Pathogenic *E. Coli.*

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ABSTRACT

The present study has been adopted to through light on the antimicrobial effect of the chitosan hydrogel and Silver nanoparticles against commercial antibiotic resistant pathogenic *E. coli* as a model of Gram negative bacteria, through diagnosis of 100 diarrheic patient samples, results obtained showing that 43 *E. coli* strain was isolated out of 100 total samples, from which 21 Out of 43 samples were proven by sensitivity test to be antibiotic resistant. The antimicrobial activity of chitosan hydrogel was investigated against *E. coli* isolates, chitosan hydrogel proposed mechanism was found to be via the attraction of sections of anionic microbial membrane into the hydrogel's surface pores causing microbial membrane disturbance and hence microbe elimination. Silver nanoparticles with concentrations ranged from (25-100 mg/L) were confirmed to be effective bactericides throughout attraction between cation Ag and anion sulfur content of *E. coli* cell wall and discourage the growth of tested *E. coli* isolates. SEM and TEM have been used for studying and analyzing the biocide action of the used Antimicrobials. The results confirmed that the treated *E. coli* cells exhibit cell death and accumulation of hydrogel and or silver nanomaterial while the used traditional antibiotics have no significant effect.

Keywords: Antibacterial activity, Antibiotic, Chitosan, *E. coli,* Hydrogel, Silver nanoparticles.

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INTRODUCTION

The spread and development of antibiotic-resistant microbes leads to increasing the difficulty of microbial infections treatment [1]. Microbial infections considered the most important causes of human life's risk as, they may cause very serious pulmonary disease [2]. Producing new bacteria resistant strains for existing antibiotics has become a vital problem in the field of public health; hereafter, there is a strong inspiration for developing new bactericides [3-4]. Bacteria has various membrane structures which can be classified as Gram-negative or Gram-positive. The structural differences in peptidoglycan component of the cell wall represent the key point for diagnoses. Gram negative bacteria exhibit only a thin cell wall membrane [5]. While, in contrast, Gram-positive bacteria lack the outer membrane but have a peptidoglycan layer with about 30-32 nm thickness [6]. Genetically *E. coli* has been considered one of the most flexible bacteria also, it is the main source of several phages mediated and plasmid genes [7]. *E. coli* causes diarrhea and septicemia for hosts. One or more virulence factors including invasiveness considered the main reasons for pathogenicity of *E. coli* [8]. Antibiotic resistant *E .coli* isolates lead to increasing in mortality, morbidity and increasing the treatment cost. Therefore, development, modification and control of *E. coli* antibiotic resistant strain is becoming a very important issue. So, the bactericidal potential against *E. coli* antibiotic resistant became a priority [9]. Due to all the mentioned, novel antibacterial are of great challenge to overcome resistance that developed from numerous pathogenic microorganisms against most of the traditional antibiotics [10]. Both Sliver nanomaterial and Chitosan hydrogel are the new trend dealing with this issue as they hold promises to kill microbes effectively [11].

Hydrogels are three dimensional networks prepared using either natural or synthetic polymers with high to high degree of water swelling. Wichterle and Lim in 1960 reported the first biological and biomedical use of hydrogels [12]. Nowadays, there is an explosion of investigations and researches verifying the use of hydrogels in different biomedical applications [13–14]. Studied extensively illustrate the accurate preparation of natural hydrogel has been done using polysaccharides as a backbone. The two polymers Chitosan and Alginate have been broadly studied in the past [15].

Chitosan (Ch) is a cationic linear polysaccharide, widely studied and used for a extensive range of biological applications based on its biocompatibility, biodegradability, haemostatic activity, antibacterial activity, anti-tumor activity, wound healing acceleration and low toxicity [16]. Grafting of acrylamide onto chitosan results in antimicrobial resin for medical application [17]. Scientists reported the Bactericidal efficiency of chitosan, where both G. negative and G. positive bacteria have been used. The antibacterial activity of chitosan may be according the positive charge of chitosan backbone which moiety can interact with bacteria with negative charge cell surface [18].

Chitosan hydrogels were synthesized and analyzed for their antimicrobial activity against, Bacillis subtilis, Aspergillus fumigatus, S. aureus, Salmonella typhimurium, A. niger, Streptococcus, and *E. coli*. Ming et al., compare the effect of chitosan and carboxymethyl chitosan hydrogel on *E. coli* and the results showed significant antimicrobial activities of the pure and treated chitosan [19]. The chitosan antimicrobial effect against G +ve and G -ve bacteria, filamentous fungi, and yeasts have been studied [20].

Chitosan-γ-poly(glutamic acid) hydrogel has been prepared, the hydrogel displayed positive antimicrobial activities against *E. coli* and S. aureus [21]. Furthermore, Chan-Park et al., prepared an antimicrobial hydrogel composed of dimethyl alkyl ammonium chitosan-graft-poly(ethylene glycol) methacrylate [22]. Subsequently, Aziz et al. described the development of an antimicrobial chitosan-dextran (CD) hydrogel for use in endoscopic sinus surgery [23]. The resultant hydrogel has been found to be positive against *E. coli,* Streptococcus pyogenes, S. aureus and Clostridium perfringens (Grampositive) at its surgical concentration of $5*10^4$ mg/L.

More recently, Mohamed et al., prepared crosslinked antimicrobial chitosan hydrogels which exert greater activity against the Gram-positive over the Gram-negative bacteria. Meaningfully, the authors noted that increasing the hydrogel crosslinking degree will cause an enhancement in the antimicrobial activity. [24].

Preceding studies apprehensive using metal nanoparticles especially those of silver and gold were found to exhibit resistance to microorganisms [25-26]. It was also stated that between different nanoparticles, nanosilver displayed adequate antimicrobial effects [27]. Silver nanoparticles are known as non-toxic

antibacterial agents because hey demonstrate a strong toxicity to a variety micro-organisms, for this reason silver nanoparticles used in the field of different biocidal applications [28]. Silver nanoparticles are able to physically interact with the cell surface of various bacteria. As, ionic silver strongly interacts with thiol groups of vital enzymes and inactivates them. Many studies have reported that silver nanoparticles can damage cell membranes leading to structural changes, which render bacteria more permeable [29]. Further studies showed that using silver nanomaterials cause structural changes in the cell membrane as well as the formation of small electron-dense granules. Silver nanoparticles have incredible antibacterial resistance to antibiotics due to the increased catalytic activity on both gram positives and gram negatives bacteria. This effect is highly influenced by the nanoparticles' size, shape in addition to concentration [30] and a study using Escherichia coli [31] confirmed that silver nanoparticles accumulation on the membrane cell creating gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death.

The eventual goals of this study may be summarized in the following points:

1- Conclude the prevalence of *E. coli* in fecal samples of diarrheic patients and establish their antibiotic sensitivity profile.
2- Developing a new non-toxic bactericides against disease causing microbes that have become resistant to drugs therapy.
3- Studying the sensitivity of *E. coli* isolates to different antibiotics widely used in veterinary field.
4- Studying the interaction between isolated E. coli from diarrheic samples with both biocidal silver nanoparticles and chitosan-graft-poly (acrylamide) hydrogel prepared using microwave irradiation.
5- Application of scanning and transition electron microscopy techniques for studding the mechanism which used by silver nanoparticles and hydrogel to interact with the isolated bacteria.

MATERIAL AND METHOD

Materials

Chitosan-medium molecular weight (400,000 g/mol, 75–85% unities deacetylate – shrimp origin) from Sigma-Aldrich Co. (St. Louis, MO, USA) and used as supplied. Analytical grade of acrylamide (Merck, Darmstard, Germany), hydrogel synthesis experiments were conducted using: ultraviolet irradiation system - wavelength 254 nm (produced from El-Gomhoria company for chemical and laboratory accessories, Egypt).

Microorganisms and Culture Conditions

A total of 100 diarrheic samples have been collected and tested for isolation of the causative microorganism of diarrhea. A loopfull from each diarrheic sample was streaked onto the surface of blood agar, nutrient agar, MacConkey's agar, EMB and Luria–Bertani (LB) medium broth. The inoculated plates were incubated at 37°C for 24-48 hours and examined for bacteriological growth. Suspected colonies, appearing on different media were sub-cultured, purified, and preserved in semisolid agar for further identification. Pure colonies were described for their morphological characters, colonial appearance and were biochemically identified according to Koneman et al., [33]. All isolates have been tested for measuring their sensitivity to antibiotics by means of the disc diffusion method [34]. Sensitivity was checked by visualizing the zone of inhibition around the disc. (All chemicals and antibiotics were supplied by Sigma-Aldrich).

Sensitivity of *E. coli* for commercial antibiotics

All isolated *E. coil* strain tested for their sensitivity against different antibiotics used in large scale by means of using disc diffusion method involved by the ceftriaxone 30 µg per disc (CRO), cefuroxime 30 µg per disc (CXM), ciprofloxacin 5µg per disc (CIP) and (CN) 5µg per disc [35]. **Table (1)** shows the common and commercial antibiotics used as antibacterial.

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Table (1) Commercial antibiotic used.

Mode of action (inhibition)	Antibiotic family	Antibiotic	Abbreviation
Cell wall synthesis	Cephalosporin 3rd generation	Ceftriaxone	CRO
	Cephalosporin 2nd generation	Cefuroxine	CXM
DNA replication	Quinolones	Ciprofloxacin	CIP
Protein synthesis	Aminoglycosides	Gentamycin	CN

Synthesis of Sliver Nanoparticles

Silver nanoparticles has been prepared using mentha piperita leaf broth as mentioned by Upendra et al., [36] with simple modifications, where, 30 mg of mentha piperita leaves were washed thoroughly using sterile distilled water and dried in open air. Leaves were finely grinded and boiled in 100 ml sterile distilled water for 30 min. Leaf broth was sterilized by filtration (0.45 µm) sliver nanoparticles are prepared by reducing 1µm Ag NO_3 aqueous solution with freshly prepared leaf broth. All procedure was performed at room temperature and under atmospheric pressure. The final manufactured powder of silver nanoparticles inside a carbon matrix, which prevents coalescence during synthesis. The obtained powder was redisposed in deionized water by sonication and therefore sliver nanoparticles at spherical shape and concentration were easily controlled to perform interaction of the silver nanoparticles with the bacteria. The silver nanoparticles in the solution are characterized by placing a drop of the homogeneous suspension in a transmission electron microscope (TEM) copper grid with a lacy carbon film and then using TEM at an accelerating voltage of 200 kV.

Synthesis of Chitosan Grafted Hydrogel

The grafting procedure has been carried out following the procedure which has been described by Marwa et al., with some modifications as follows [34]. 4 g chitosan has been added to 20 mL of acidified distilled water. The mixture was stirred at 350 rpm for 24 h at room temperature. Chitosan solution were then being subsequently added into 2 g acrylamide, 0.7 g potassium persulfate (KPS) and 0.3 g methylene bis-acrylamide (MBA) in a 250-mL reactor equipped with magnetic stirrer, and stirred for 10 min. then, Then, grafting step has been done using UV irradiation technique for 60 min. The reaction product has been allowed to cool to room temperature. Finally, the product was filtered to remove undissolved particles, washed twice with fresh ethanol and dried at 70 °C till constant weight was achieved. The produced hydrogel has been grinding to a size ranged between 20-30 µm.

Hydrogel UV-sterilization

To sterilize the prepared hydrogel samples, UV illumination under a universal UV light source (G36T5L/C, 254 nm, 42 W, NuAire Inc., Plymouth, MN) in a Biological Safety Cabinet (Model NU-425-600, Class II, type A/B3, NuAire Inc., Plymouth, MN) was used for 30 min for each side of the specimens. Then, the sample has been transferred into sterile polystyrene test tubes (17 × 100 mm, Evergreen Scientific, Los Angeles, CA) containing 5 mL sterile LB medium. The tubes containing the hydrogel were incubated with shaking at 200 rpm at 37 °C overnight. [38]

Hydrogel Swelling Behavior

SWR is generally used to describe the swelling behavior of the prepared hydrogel. The dry hydrogel was soaked in DW and different buffer solution over 6 hr time period at room temperature. The swollen samples have been allowed to drain using a plastic strainer for 10 min then; it has been weighed. SWR is given by the following equation [39]

$$SWR\ (g/g) = \frac{(W_s - W_d\ )}{W_d}$$

Where: W_s and W_d represent the weight of the wet and the dry hydrogel, respectively.

Antimicrobial Assessment

Antimicrobial test has been done for the prepared *E. coli* bacteria using both chitosan hydrogel and silver nanoparticles. In addition, for testing antimicrobial effect for hydrogel and nanoparticles, four different types of antibiotics have been tested. The range of the used concentrations for each antimicrobial tested are given in Table (2).

Table (2): The concentrations range of different antimicrobial tested

Antimicrobial type	Concentration (µg/L)
Chitosan hydrogel	5, 10, 15, 20 and 25 (*10^{-6})
Silver nanoparticles	0, 25, 50, 75 and 100
Ceftriaxone	32, 16, 8, 4 and 2
Cefuroxine	64, 32, 16, 8 and 4
Ciprofloxacin	32, 16, 8, 4 and 2
Gentamicin	32, 16, 8, 4 and 2

Experimental Procedure using Silver Nanoparticles

Different concentrations of silver nanoparticles (0, 25, 50, 75 and 100 mg/L) were tested against *E. coli* bacteria. Agar plates with different concentrations of silver nanoparticles were prepared, followed by the plating of a 10 µl sample of a culture media with an optical density of 0.5 at 595 nm and 37 ^{0}C. The interaction with silver nanoparticles was investigated by growing bacteria at an optical density at 595 nm of approximately 0.5 at 37 ^{0}C in LB culture medium. Then, silver nanoparticles were added to the solution, making a homogeneous suspension of 100 µg/ml and leaving the bacteria to grow for 30 min. The cells are centrifugated (3000 rpm, 5 min, 4°C), washed and then suspended using a PBS buffer solution. A 10 µl sample drop was deposited on TEM copper grids covered with carbon film and the grid was then exposed to glutaraldehyde vapors for 3 min. to fix *the E. coli* isolates. TEM analysis using sample staining was also carried out. The sample provision monitored by the same procedure as the cross-sectioned sample slices but before the dehydration process the cells were tinted with a 2% OsO_4 /cacodylate buffer for 1 hr.

Silver detection electrochemically at low concentrations, it is essential to electro-deposit silver against the electrode surface in a pre-concentration step by holding the potential of the electrode at −0.3 V versus Ag/AgCl for 60 s. This procedure reduces Ag+ to Ag0, which plates against the electrode surface. When the potential is swept positively from −0.3 to +0.35 V, the deposited silver is oxidized to Ag+ and coated on the electrode, giving a characteristic stripping peak with a height proportional to the concentration of Ag+ in the solution.

RESULTS AND DISCUSSION

Bacterial infectious causing diarrhea have been attributed to enteropathogenic *E.coli* .The pathogenic *E.coli* stick to the mucosa and multiply in the lumen of intestine, producing a potent enterotoxin, which stimulate extreme secretion of fluid from intestinal mucosa. This loss of fluid causes the principal sign (diarrhea) and often leads to dehydration [40].

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Table (3) Prevalence of *E. coli* isolated from diarrheic samples

Total No. of samples	Species	# +ve samples for *E. coli*	%
100	*E. coli*	43	43

In the current study, 43 samples out of 100 were positive to bacteriological examination with an incidence of 43%. All isolated *E.coli* were identified using the standard test recommended for the identification of the isolated *E. coli*, higher incidence of isolation were acceptable if compared with that obtained by study performed in Italy moiety.

It is essential before discuss our results to shed light on the significance of occurrence of diarrhea due to *E. coli* with this moderately high incidence. The present study focused mainly on two main issues, first, non-disciplined and random use of antibiotics which are translated to the high resistant rate . So, which become necessary for paying attention to the necessity of using a new tool that can overcome the effects of which unsatisfactory to antibiotics which certainly needs additional researches.

The sensitivity to antibiotics was estimated according to (oxoid). Results concerning sensitivity test are summarized in **Table (4)** showing that 21 strain out of 43 were positive *E.coli.* The isolated strains were resistant to all tested antibiotics at different concentrations used. Isolates responded intermediately to the antimicrobial effect of (CN) antibiotic. The aforementioned results of antibiotics resistance from our point of view came forth because of too frequent and inappropriate use of antibiotics and our results agree with that mentioned by (29) who described that a growing number of bacterial species are simultaneously becoming resistant to more than one antibiotics.

Table (4) Sensitivity of *E. coli* isolates to different antibiotics

No. of isolate	Organism	CN	CRO	CXM	CIP	# *E. coli* Resistant& intermediate resistance
43	*E. coli*	1 I	9 R	8 R	3 R	21 –

Where: R: Resistant & **I:** Intermediate

Evaluation of antimicrobial activity of Chitosan hydrogel

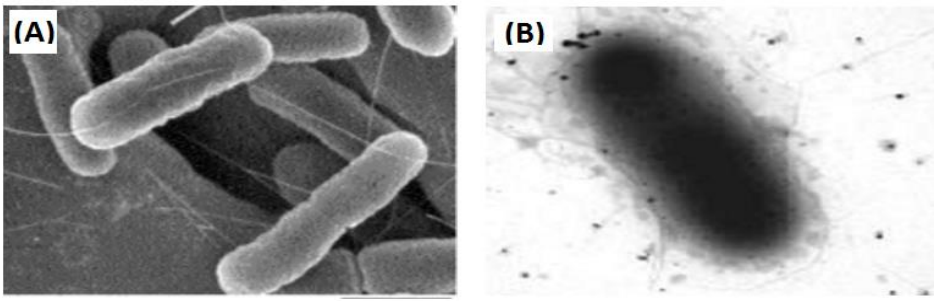
E.coli strains were inhibited after a period of more resistance by hydrogel at concentrations 50,000 mg of hydrogel/liter.

Hydrogel Swelling Behavior

Swelling ratio shows the superabsorbent ability of the prepared hydrogel on absorbing water. The water swelling study of chitosan-graft-acrylamide superabsorbent hydrogel from this experiment is shown in **Figure (1)**. Examination was performed after 6 hr soaking time as from our previous work the swelling process is increased until 6 hr then the rate is slow down and almost being constant (MM), in various quantities of chitosan in the reactant mixture (2; 3; 4; and 5 g). It can be noted that during the first five hours, the water swelling rate increased sharply and after that begins to stop which may be attributed to the fact that the increase in the water uptake capacity in begging level may attributed to the monomer molecules availability in the vicinity of the chain propagating sites of polysaccharide backbone (BA). The addition of chitosan in the reactant composition cause

the decreasing of water swelling ratio. The water absorbency was getting lower, SWR was decreasing by the increasing the chitosan concentration. This is due to the excess of chitosan which can act as IPN (Interpenetrating Network), fill in the polymer matrix and cause some difficulties for water to penetrate. The highest swelling capacity of 99 (g water/g dry hydrogel) was reached at 24 hours, resulted from hydrogel sample 1 with 2 g chitosan.

Figure (1) Effect of pH on swelling water ratio for the prepared Chitosan hydrogel



Motility of the isolated *E. coli* strains

The motility of *E. coli* pretreated with 16,000 µg of C. hydrogel /L was reduced (score = 2). After pretreated with 32,000 µg of C. hydrogel /L, growth extended only slightly beyond the inoculation line score =1). The apparent inhibitory effect since the identical results were achieved in medium without the presence of dye.

E. coli displayed movement to the MacConkey agar plug, surrounding it after 24 h incubation. Pretreatment with 16,000 µg slowed the rate motility of *E. coli* to some extent, whereas at a concentration of 32,000 µg/L the bacterial motion was minimal in that movement away from the inoculation point occurred nonetheless the bacteria failed to surround the nutritive plug after incubation for 24 h. In all the experiments it was observed that there was no movement toward the non-nutritive plug.

The used chitosan hydrogel sample have a bactericidal effect against *E. coli* at or below a concentration of 50,000-µg/L. The antibacterial activity of Hydrogel was probably primarily due to their action of the aldehyde groups with amino groups in proteins located in cell membranes [41], nevertheless may be attributed to the breakage of the peptide cell wall peptidoglycan bonds. Aldehyde-containing biocides, such as ortho-phthalaldehyde and glutaraldehyde, are reported to react with primary amino groups located on the outer envelope or cell wall, producing a strong adhesive effect [42]. Chitosan hydrogel has resistance to *E. coli* which may be attributed to some features for example: low permeability of the cell wall, chromosomal mutation and genetic capability to express resistance mechanisms [43].

The tested strain was susceptible to hydrogel in which the reaction between negatively charged aldehyde groups of chitosan hydrogel with positively charged amine groups present in the chemical structures of *E.coli* cell membrane. The integrity of *E. coli* membranes was also examined by measuring the release of intracellular components by measuring absorption values at 260 and 280 nm via UV-vis spectrophotometry. The results obtained in this experiment was not conclusive because hydrogel by itself absorbed at both 260 and 280 nm.

TEM and SEM have been used to study the cellular effects of hydrogel on isolated *E.coli*. Increased cell aggregation was detected for E. coli cell with 32,000 mg hydrogel/L caused cracks in the cell wall in addition to clumped flagella and as lightly shrunken appearance compared to the untreated *E. coil* “control sample”. TEM of untreated *E. coli* control cells revealed an even distribution of cytoplasm and good membrane integrity as seen by electron-dense lines. For helping in clarifying the mechanism of this process, information on the morphology of *E. coli* following treatment with hydrogel was required using electron microscopy. SEM images revealed cell wall damage compatible with the mechanisms of action proposed above as shown in Figure (2). As shown in Figure (3) TEM images of the bacteria treated with hydrogel were quite similar to those obtained with

the SEM . The action primarily involves binding of the hydrogel component with amino groups in proteins located in the bacterial cell wall.

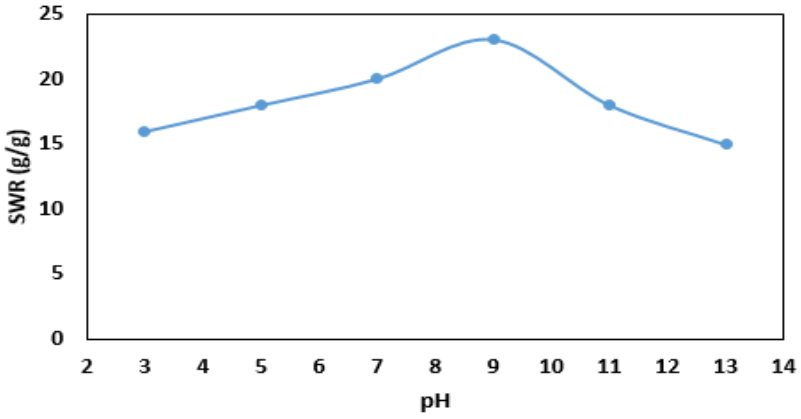
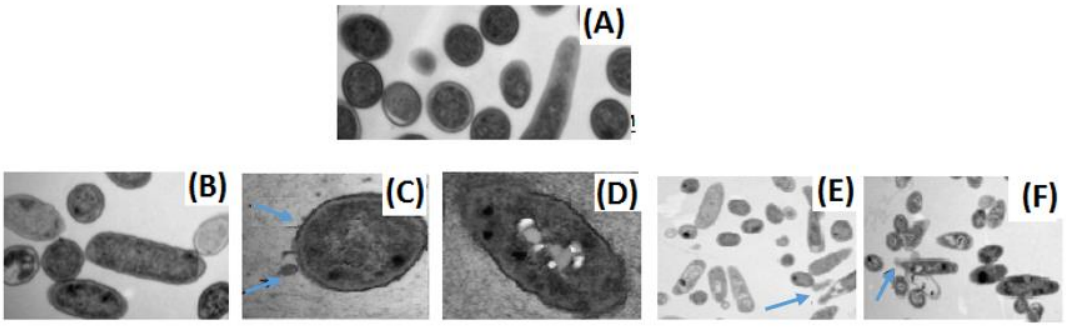


Figure (2) SEM (a) untreated *E. coli* (b) treated *E.coli* incubated with 32,000 µg of hydrogel/liter

Figure (3): (A) TEM effect of membrane ultra structure and intra cellular distribution Untreated *E. coli*. .



(B to F) *E.coli* treated with 0.02%Triton X-100 (B), 32,000 µg /liter (C and D), 16,000 µg liter (E), or 32,000 µg /liter (F). Blue arrows indicate blabbing.

Effect of Silver nanoparticles on *E. coli*

Bacteria was tested in the presence of silver nanoparticles with different concentrations to observe the effect on the bacterial growth. The results indicated that increasing the concentration of silver nanoparticles will prevent bacteria growth, at concentrations above 75 µg ml^{-1} there was no significant growth detected for *E. coli.*

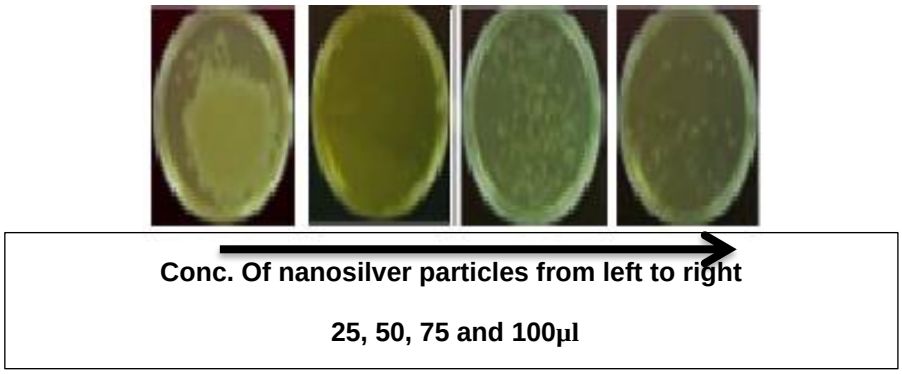


Figure (4) Bacteria grown on agar plates at different concentrations of silver nanoparticles, SEM images that show the interaction of the bacteria with the silver nanoparticles: *E. coli.*

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SEM analysis of the polymerized sample showed the interior of the bacteria. It demonstrated that the nanoparticles are not only on the surface of the cell membrane, but also exits inside the bacteria (Figure 5 (a-b). This results have been confirmed using an elemental mapping analysis using the x-ray energy dispersive spectrometer (EDS) in the TEM (Figure 5(a)). The nanoparticles were found distributed all throughout the cell; they were attached to the membrane and were also able to penetrate the bacteria.

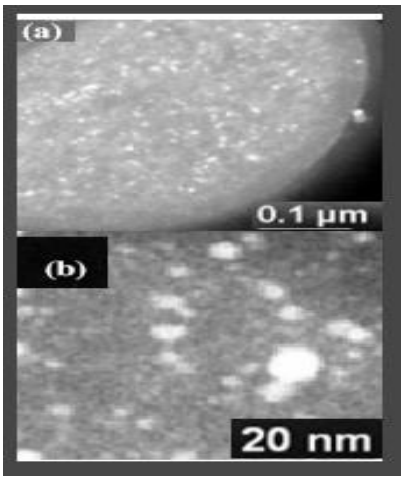

Figure (5) (a) a considerable presence of silver nanoparticles is found in the membrane and the inside of an *E. coli* sample. (b) *E. coli* sample treated with silver nanoparticles.

A different sizes of nanoparticles distribution in the interacting with *E. coli* has been determined as indicated in Figure (5). The main size of these silver nanoparticles was about 5 nm (± 2 nm). While, it was found that the silver nanoparticles with different particles sizes were affecting directly *E. coli*. This distribution corresponds to the lower end of the size distribution for the released silver nanoparticles. It can be noted that the bactericidal is highly effected by the silver nanoparticles size.

TEM mode were very like those of SEM proved that the obtained results depending on the morphologies of the bacteria in addition to the effects of the particles with the bacteria in as shown in Figure 5(a & b). The silver nanoparticles were located in the membrane of the bacteria along with in onside it.

SEM analysis was used to study the surface morphology of both native and treated *E.coli*. In which non treated *E.coli* cells showed straight rods occur singly, on the other hand *E.coli* cells after treatment by sliver nanoparticles were significantly changed and showed major damage. That was characterized by the formation of " pits" in their cell walls, exit of the components of some cells and decreasing number of bacterial cells. On the base of membrane structures, bacteria may be classified as: Gram negative and Gram positive. The structural difference lies in the organization of peptidoglycan, which is the key component of membrane structure. Gram-negative bacterium displays a thin layer of peptidoglycan (about 2–3 nm) between the cytoplasmic membrane and the outer cell wall. Outer membrane of *E.coli* cells is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provides an effective permeability barrier.The mechanism by which the nanoparticles are able to penetrate the bacteria membrane surface is not totally clear, but Salopek previous suggests that when of *E. coli* treated with silver nanoparticles the modifications created in the membrane morphology may produce a significant increase in its permeability and hence affect proper transport through the plasma membrane[4]. In the present study, method could explain the significant numbers of silver nanoparticles found inside the bacteria. The reflection of silver nanoparticles attached to the cell membrane and inside the bacteria (Figures 5(a &b)) is fundamental in the understanding of the bactericidal mechanism. The membrane content of the bacteria is sulfur-containing proteins; these might be superior sites for the silver nanoparticles. Moreover, nanoparticles found inside will also tend to react with other sulfur-containing proteins in the interior of the cell, as well as with phosphorus-containing compounds such as DNA. The changes in morphology presented in the membrane of the bacteria, as well as the possible damage caused by the nanoparticles reacting with the DNA, will affect the bacteria in processes such as the respiratory chain, and cell division, finally causing the death of the cell [44] .

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To determine the antibacterial effects of sliver nanoparticles and chitosan hydrogel on the isolated E.coli strain, where used imipenem (IP) as a standard for *E.coli* bacteria PRIMAXIN (2009). According to the pervious reference the following scales were used in the study. Weak effect of sliver nanoparticles (≤ 10 mm) in diameter. Intermediate effect (>10 to 14 mm) in diameter. High effect of sliver nanoparticles (> 14 mm) in diameter for G –ve *E.coli*.

The result of E.coli were summarized in Table (5) for sliver nanoparticles. All tested isolates which showed resistance to different antibiotics exhibit sensitivity toward the treatment by using nanoparticles.

Table (5) Detection the bactericidal effect of sliver nanoparticles.

No. of isolates	Strain	Zone of inhibition (diameter mm) Sliver Nano.	Silver Nano.
21	*E.coli*	21 / S	BC

Where: S = > 14 mm in diameter; R= ≤ 10 mm in diameter; BC Bactericidal effect.

Detection of the bactericidal effect for both silver nanoparticles and chitosan hydrogel (BC) were used as antimicrobial against *E.coli* causing diarrhea , re inoculate the bacterial cells treated with both antimicrobials from zone of inhibition on nutrient agar were performed. Results revealed that sliver Nanoparticles and hydrogel gave BC effect against the all tested 21 *E.coli* isolates and no growth can exhibited.

Pathogenicity of *E.coli* isolated from diarrheic samples

Five isolates randomly choose from twenty one *E.coli* strain recovered from diarrheic samples showed antibiotic resistant's, were tested for pathogenicity in vivo using mice before and after *E.coli* treatment by both antimicrobials used in the present study.

Before and after use of antibiotics on *E.coli* strains, it was found that out of 5 isolates, 5 (100%) proved to be fatal to mice on intra-peritoneal inoculation of whole bacterial culture.

After using silver Nanoparticles and hydrogel on E.coli isolates, it was find a fantastic result as out of 5 isolates ,0 (0%) proved to be not fatal to mice on intra-peritoneal inoculation of whole treated E.coli bacterial culture. And no bacterial growth on agar for treated *E.coli*. Nearly same results were recorded by Deshpande et al., [45] they mentioned that twenty seven isolates of *E. coli* recovered from diarrheic samples were tested for virulence factors by a battery of in vivo (pathogenicity and enter toxigenic) and in vitro methods. These results was confirmed by our results in which all treated *E.coli* by silver nanoparticles were damaged in their cell wall and finally death.

CONCLUSION

It is concluded that, according to the development of antibiotics resistance and the outbreak of infectious diseases caused by resistant pathogenic bacteria, most of scientists searching for new unconventional antibacterial agents. The bacterial strains which were resistant to antibiotics may be highly susceptible to silver Nanoparticles and new trend hydrogel treatment. So, the use of Ag NPs and chitosan hydrogel, could be effective antibacterial agent against these multidrug resistant strain of bacteria.

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