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Enhancement of Antimicrobial Sensitivity of *Salmonella* and *Escherichia coli* Strains Isolated from Chickens Using Silver Nanoparticles in Assiut Governorate

Moemen A. Mohamed[1*], Fatma M. Mohamed[2] and Waleed A. El-Said[3]
[1]Poultry Diseases Department, Faculty of Veterinary Medicine, Assiut University, Egypt
[2]Poultry Diseases Department, Animal Health Research Institute, Assiut Regional Laboratory, Egypt
[3]Chemistry Department, Faculty of Science, Assiut University, Egypt

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Abstract

Before the era of complete resistance to antibiotics due to their extensive use in poultry farms, new strategies were discovered, one of them was the use of nanoparticles to enhance the action of antibiotics. Therefore, this study was carried out to find out the antibacterial effect of silver nanoparticles (AgNPs) either separately or in combination with antibiotics. The obtained data showed the antibacterial activity of AgNPs against the tested *Escherichia coli* and *Salmonella* strains with MIC level of 0.85 µg/mL. Synergistic effects of AgNPs with antibiotics against *E. coli* revealed two-fold drop in MIC of ciprofloxacin and amoxicillin. Meanwhile, there was three-fold drop in MIC of gentamicin, cefotaxime and neomycin. Significant finding was observed in the case of the synergism of AgNPs with amoxicillin and gentamicin, the examined *E. coli* O2 resistant to amoxicillin and gentamicin became sensitive when the antibiotics were combined with AgNPs. It could be concluded that AgNPs can be easily produced by Rosemary aqueous extracts as low-cost, eco-friendly method for generating AgNPs. New generations of bactericidal compounds containing AgNPs could be successfully used in poultry farms for prevention and treating *E. coli* infections.

Keywords: Nanoparticles, *E. coli*, *Salmonella*, Antibiotics, Chickens

Introduction

Bacterial infections in commercial poultry farms cause significant losses in poultry industry. There are different classes of bacteria, which are distributed in commercial broiler chicken farms. Among these, *Escherichia coli* and *Salmonella* species are associated with diverse manifestations in broiler chickens and are considered important foodborne pathogens to human beings [1].

Antimicrobial resistance is one of the most common serious threats facing poultry industry. The control of antibiotics resistance appears to be decisive to maintain high income and condense losses [2]. This problem of antimicrobial resistance pushed the scientists to search for new alternatives to antibiotics.

Nanotechnology is rising as a rapidly mounting branch with its application in manufacturing and science [3]. Nanoparticles are groups of particles with size ranged from 1–100 nm where the nano is one billionth of meter (10^9). This size of nanoparticles results in different characters than those of the materials of large particles. The unique properties of nano material may due to their smaller size and quantum dot. The nanoparticles of metals such as silver, copper, zinc, and gold have a bactericidal feature [4,5]. Silver nanoparticles (AgNPs) have been confirmed to be an effective antimicrobial agent against bacteria, viruses and other micro-organisms [6,7].

AgNPs could be obtained by different techniques such as physical, chemical or biological methods. Biological conversion methods could be done using microorganism, enzymes and plant extract, and at the same time is safe and eco-friendly as it does not use harmful chemicals for the conversion of silver nitrate particles to nanoparticles in comparison to other methods [8,9].

Feng *et al.* [10], Percival *et al.* [11] and Sondi and Salopek-Sondi [12] investigated the antibacterial effect of silver nanoparticles against different classes of microorganisms such as *E. coli* and *Staph. aureus*. The

***Corresponding author e-mail: (moemenmohamed@aun.edu.eg), Poultry Diseases Department, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.**

mechanism of the antibacterial action of AgNPs is due to the attachment of the particles to the surface of the cell membrane, resulting in disruption of membrane permeability and the cell respiratory functions [13].

In broiler production, there is a great need to minimize the degree of bacterial resistance against many used antibiotics. Using AgNPs alone may act as a substitute to antibiotics, while, their use in combination with antibiotics results in synergy of their power against bacteria. The present study, therefore, aimed to estimate the effect of AgNPs application either alone or in combination with commonly used antibiotics on the reduction of MIC levels of these antimicrobial agents and converting the tested *Salmonella* and *E. coli* strains from the resistance status to sensitive one.

Material and Methods

Samples

Sampling was carried out from July-2016 to November -2016 in ten broiler chicken farms located in Assiut City, Egypt, with birds aging 27 to 35 days. For Salmonella isolation, 10 individual cloacal swabs were randomly collected from each farm from birds with respiratory, intestinal and/or locomotor signs. The samples were then transported in 1.5 mL tubes containing 750 µL of Brain Heart Infusion (BHI) broth refrigerated in ice box to the Laboratory of Poultry Diseases department, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. For *E. coli* isolation, 10 liver samples were collected separately from freshly dead birds at each farm then transported refrigerated in ice box to the laboratory for finalizing the steps of isolation.

Isolation and Identification of the suspected bacteria

For the isolation of *Salmonella* spp., the following method was used in brief: BHI broth tubes were incubated at 37ºC for 18 hours then a loopful was transferred to Rappaport-Vassiliadis broth then incubated at 37ºC for 24 hours. Samples were streaked on Brilliant Green agar with Novobiocin (40 µg/mL) and *Salmonella-Shigella* agar and incubated for 24 hours at 37ºC. After incubation, colonies from each sample with characteristic morphology were subjected to biochemical identification [14]. Isolates with biochemical profile compatible with *Salmonella* spp. were identified serologically using antisera (Difco) in agglutination tests on the basis of somatic O antigen and phase 1 and phase 2 flagella antigens according to the Kauffmann-White scheme.

For isolation of *E. coli*, the surfaces of collected livers were seared with hot spatula then a loopful was taken from each liver sample and placed in BHI at 37ºC for 18 hours. A loopful of the incubated BHI broth was then streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Colonies with characteristics morphology (dark colored colonies with a brilliant green sheen were selected and identified with biochemical reaction [14]. After that, the suspected strains were divided into two parts. The first part was used for serological identification and the second part for carrying out the antimicrobial resistance for the selected antibiotics and the effect of nanoparticles in reducing the resistance of bacteria to the examined antimicrobial agents.

Biosynthesis of silver nanoparticles

Aqueous extracts (10%) of *Rosmarinus officinalis* (rosemary) were made for the preparation of the silver nanoparticles as the following: ten grams of dry ground rosemary powder were dissolved in 100 mL distilled water then heated at 80°C for one hour. After cooling by 1 hr, the extract was filtered with Whatman filter paper no 1.

The silver nitrate ($AgNO_3$) (AR grade ≥ 99.9% purity, Sigma-Aldrich) was used as a source for silver nanoparticles by adding 169.3 mg of silver nitrate (0.01M) to the 100 mL of rosemary aqueous extract in a dark container to diminish photo-activation of silver nitrate for 24 hours at room temperature. The synthesis of silver nanoparticles was indicated by the color change in the solution, from colorless to yellowish brown [15].

The UV absorption of the prepared sample was recorded by using UV-Vis which is spectroscopy double beam PC Scanning Spectrophotometer UV Evolution 300 from lambomed. A computer data system is UV Win5 software v 5.0.5 used for measuring wavelength and absorbance.

Spectrophotometer ranges from 200 to 900 nm using 1 cm matched Stoppard quartz.

Atomic absorption spectroscopy

The concentration of the silver nanoparticles was measured based on atomic absorption technique by using absorption technique spectrophotometer (Buck model 210 VGP, Buck Scientific Inc. East Norwalk, CT, USA).

The conversion of Ag to nanoparticles was carried out following the method described by Sharma *et al*. [16]. Briefly, by centrifugation of one mL of the sample at 14.000 rpm, the unreacted silver nitrate (Ag^+ ions) were in the supernatant because Ag ions are much smaller than Ag nanoparticles (Ag^0) and the formed pellets contained the Ag nanoparticles (Ag^0). The obtained supernatant solution was then analyzed by AAS to detect the amount of Ag^+ ions. The decrease in the concentration of the Ag^+ ions notifies the conversion of Ag^+ to Ag^0.

Transmission Electron Microscope (TEM)

The morphology and sizes of silver nanoparticles were determined in the 3rd concentration by TEM micrographs using the JEOLTEM100CXII (Electron Microscope Unit, Assiut University, Egypt). The sample was prepared by placing a drop of synthesized silver nanoparticles on a negative carbon coated copper grids and dried in air [17].

Detection of minimum inhibitory concentration (MIC)

The antimicrobial effect of AgNps, amoxicillin, cefotaxime, ciprofloxain and gentamicin was checked in microtiter plate 96 wells using double fold micro-dilution method against *E. coli* O1, O2, O78 and *S.* Typhimurium and *S.* Enteritidis in a density of 10^5 CFU equal to 0.1 absorbance on OD 625 [18]. The concentration of each antibiotic started from 512mg/L and biosynthesized silver nanoparticles begin with 13.6 µg/mL (13.6 mg/L) using two-fold serial dilution in Mueller–Hinton (MH) broth (100 µL in each well).

To each concentration, 100 µL of 10^5 CFU/mL of the tested microorganisms (*E. coli* O1, O2 and O78, Typhimurium and *S.* Enteritidis) were added in a final volume of 100 µL. The bacterial inoculum broth was taken as a positive control and another containing either Ag nanoparticles or antimicrobials broth without bacterial inoculums was considered as a negative control. The microtiter plates were incubated at 37ºC for 24 hours and examined for the lowest concentration showing no detectable growth (MIC). The breakpoints of sensitivity and resistance for *Enterobacteriacea* **(**including *Salmonella* and *Shigella* spp. are as follows: amoxicillin (S: < 8 and R: > 8), neomycin (S: < 32 and R: > 32), gentamicin (S: < 4 and R: > 16), ciprofloxacin (S: < 1 and R: > 4) and cefotaxime (S: < 1 and R: > 4) [18].

Combination effect of silver nanoparticles with the used antibiotics

Silver nanoparticles were mixed with each antibiotic solution (50 µL, each) and different concentrations were prepared with the microorganisms (100 µL of 5×10^5 CFU/mL) in a final volume of 200 µL as above using the double fold serial dilution in the 96-well microtiter plates. The plates were incubated and tested for MIC.

To evaluate the effect of the combinations, the fractional inhibitory concentration (FIC) was calculated with the following equation: FIC of antibiotic or silver nanoparticles =MIC in combination divided on MIC of the antibiotic or the silver nanoparticles alone [19]. The FIC index (FICI), calculated as the sum of each FIC, was interpreted as follows: FICI≤0.5, synergy; 0.5≤FICI<1, partial synergy; FICI=1, additive; 2≤FICI<4, indifferent; 4<FICI, antagonism [20,21].

Results

Isolation and serotyping

Out of 100 examined samples, *E. coli* were recovered from 45%. In contrast, Salmonella isolates were isolated from 5% of individual cloacal swab samples. According to serotyping, the majority of the isolated *E. coli* belonged to O78 (23 out of 45 positive strains), followed by O2 (12 out of 45 *E. coli* strains) and O1 was recovered from 10 out of the 45 strains. However, 3 out of 5 positive *Salmonella* isolates were *S.* Typhimurium, while, the remaining 2 were *S.* Enteritidis.

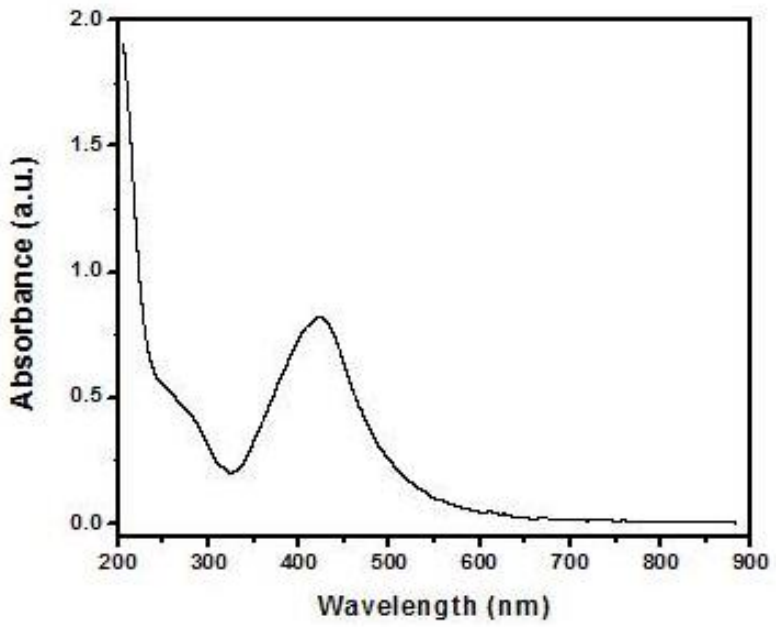

Figure 1: UV-VIS absorption spectrum of the obtained silver Nanoparticles.

Characterization of nanoparticles

Absorption spectra of silver nitrate nanoparticles formed in the reaction media has absorbance peak at 420 nm (Figure 1). Atomic absorption spectroscopy was determined by the concentration of the AgNPs at 13.6 ug/mL through a decrease in concentration of Ag ions from 1690 µg/mL to 1676.4 µg/mL indicating the conversion of unreacted silver (Ag+) to the converted silver nanoparticles (Ag0).

ATEM image of the prepared silver nanoparticles is shown in Figure 2. The Ag nanoparticles are spherical in shape with smooth surface morphology. The diameter of the nanoparticles ranged from 7.55 nm to 16.5 nm. TEM image also shows that the produced nanoparticles are more or less uniform in size and shape.

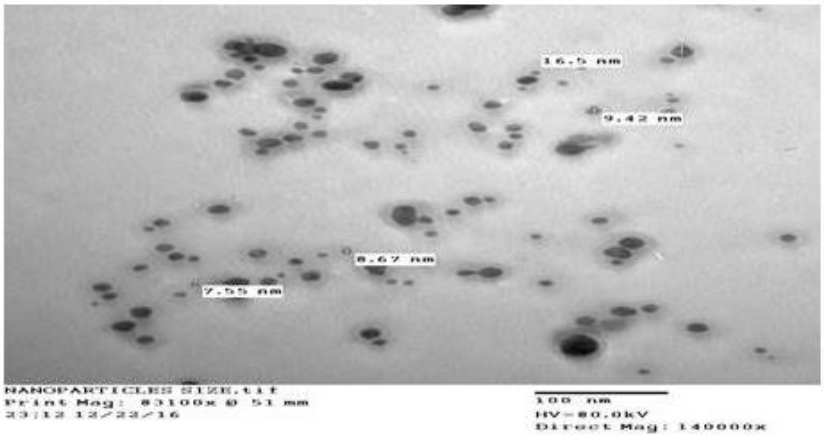

Figure 2: Transmission Electron microscope (TEM) image showing Ag-Nps formed by rosemary

Minimum inhibitory concentration (MIC) determination

For all the tested strains of *E. coli* and *Salmonella* species, the MIC of the prepared silver nanoparticles alone were 0.85 µg/mL.

The antibacterial activity of the used antibiotics

Ciprofloxacin

Table 1 shows the antibacterial activity of ciprofloxacin alone /or in combination with silver nanoparticles. The observed MIC of ciprofloxacin with the examined strains for *E. coli* and *Salmonella* strains showed resistant breakpoints levels either alone or even in addition of silver nanoparticles but have a minimizing effect on the breakpoints, but could not reach to the sensitive breakpoint levels.

However, the FIC index showed synergy in case of *E. coli* O2, partial synergy in case of *E. coli* O1, indifference in case of *E. coli* O78 and *S.* Enteritidis, but it was additive in case of *S.* Typhimurium.

Amoxicillin

As shown in Table (1), two strains of *E. coli* O1 and O2 were converted from resistant strains with breakpoint 16 mg/L to susceptible isolates with breakpoint 8 and 4 mg/L, respectively, after the addition of silver nanoparticles. In case of *Salmonella* strains; *S.* Enteritidis became more sensitive to amoxicillin with breakpoint 8 mg/L before addition of silver nanoparticles and reached to 4 mg/L after addition of silver nanoparticles. In contrary, *S.* Typhimurium strains were converted from sensitive to resistant with breakpoint of 32 mg/L. However, the FICI showed synergy in case of *E. coli* O2 and *S.* Enteritidis, additive effect in case of *E. coli* O78 and antagonism with *S.* Typhimurium.

Table 1: Antibacterial activity of ciprofloxacin and amoxicillin alone and in combination with silver nanoparticles.

Ciprofloxacin

Microorganisms	MIC			FICI	Effect
	Ciprofloxacin alone	Combination			
	(mg/mL)	(µg/mL)	(mg/mL)		
***Escherichia coli* O1**	16	0.2125	8	0.75	Partial synergy
***Escherichia coli* O2**	64	0. 2125	8	0.375	Synergy (S)
***Escherichia coli* O78**	128	0.85	32	1.25	Indifferent
***Salmonella* Typhimurium**	32	0.425	16	1	Additive (A)
***Salmonella* Enteritidis**	64	0.85	32	1.5	Indifferent

Amoxicillin

Microorganisms	MIC			FICI	Effect
	Amoxicillin alone	Combination			
	(mg/mL)	(µg/mL)	(mg/mL)		
***Escherichia coli* O1**	16	0.2125	8	0.75	Partial synergy
***Escherichia coli* O2**	16	0. 1062	4	0.375	Synergy (S)
***Escherichia coli* O78**	32	0.425	16	1	Additive (A)
***Salmonella* Typhimurium**	8	1.7	32	6	Antagonism
***Salmonella* Enteritidis**	8	0. 1062	4	0.375	Synergy (S)

S: Synergistic. A: Additive. I: Indifferent.

Gentamycin

According to that shown in Table 2, all the examined strains of *E. coli* were resistant to gentamicin where the breakpoints ranged from 32-64 mg/L. After addition of the silver nanoparticles, the MIC breakpoints of gentamicin were lowered to 8 and 4 mg/L for O1 and O2 strains, respectively, but O78 was not affected. In case of *S.* Typhimurium and *S.* Enteritidis and after the addition of silver nanoparticles, the levels of MIC breakpoints became higher than that observed with the antibiotic alone. FIC index showed synergy in

case of *E. coli* O1 and O2, indifference in case of *E. coli* O78 and *S.* Typhimurium, but it showed antagonism in case of *S.* Enteritidis.

Cefotaxime

The recorded MIC levels of cefotaxime alone showed resistance and ranged from 32 to 512 mg/L for *E. coli* and 2 to 128 mg/L for *Salmonella* strains as shown in Table 2. In case of addition of silver nanoparticles to the cefotaxime, the MIC levels were lowered but the examined isolates were still resistant and the MIC ranged from 4- 128 mg/L for *E. coli* and *Salmonella* strains. The FIC index showed synergy in case of *E. coli* O2, indifference in case of *S.* Enteritidis, additive in case of *E. coli* O1, but it showed antagonism in case of *E. coli* O78 *and S.* Typhimurium.

Neomycin

The observed MIC breakpoints of neomycin alone showed sensitivity (32 mg/ml) against *E. coli* O2 and O78, and resistant breakpoint 64 mg/mL for O1 (Table 3). However, *Salmonella* species recorded the sensitive level 16 mg/mL for *S.* Typhimurium and resistant breakpoint 64 mg/mL for *S.* Enteritidis. In case of AgNPs addition to the antibiotics, the FICI showed synergistic effect with neomycin against O1, O2 and *S.* Enteritidis, but it showed antagonism in case of *E. coli* O78 and *S.* Typhimurium.

Table 2: Antibacterial activity of gentamicin and cefotaxime alone and in combination with silver nanoparticles

Gentamicin					
Microorganisms	**MIC**			**FICI**	**Effect**
	Gentamicin alone	**Combination**			
	(mg/mL)	**(µg/mL)**	**(mg/mL)**		
***Escherichia coli* O1**	64	0.2125	8	0.375	Synergy (S)
***Escherichia coli* O2**	32	0. 1062	4	0.315	Synergy (S)
***Escherichia coli* O78**	64	1.7	64	3	Indifferent
***Salmonella* Typhimurium**	2	0. 1062	4	2.12	Indifferent
***Salmonella* Enteritidis**	8	0. 85	32	5	Antagonism

Cefotaxime					
Microorganisms	**MIC**			**FICI**	**Effect**
	Cefotaxime alone	**Combination**			
	(mg/mL)	**(µg/mL)**	**(mg/mL)**		
***Escherichia coli* O1**	512	0.85	32	1.0625	Additive
***Escherichia coli* O2**	32	0. 1062	4	0.25	Synergy (S)
***Escherichia coli* O78**	256	3.4	128	4.5	Antagonism
***Salmonella* Typhimurium**	128	3.4	128	5	Antagonism
***Salmonella* Enteritidis**	2	0. 1062	4	2.125	Indifferent

S: Synergistic. A: Additive. I: Indifferent.

Discussion

Avian collibacillosis and salmonellosis are considered to be the major bacterial diseases in poultry industry worldwide. In addition, collibacillosis and salmonellosis are the most common avian diseases that are communicable to humans [22]. In the last decade, it is well known that bacterial antimicrobial resistance is an increasing threat against elimination of bacterial infections particularly in broiler farms.

A substitute to overcome the drug resistance of microorganisms is immediately needed. Silver (Ag) has been used as antimicrobial agent against microorganisms [23]. However, there are some restrictions in using Ag salts as antimicrobial agents, therefore, using silver in nano form could be a solution.

For the assessment of the antimicrobial effects of AgNPs, *E. coli* and *Salmonella* strains were used in this study. The effect was investigated by MIC microdilution method.

Our results revealed that the bacterial growth was completely inhibited in the presence of AgNPs on the 0.85 µg/mL. It appears that these particles are bactericidal at low concentration. In accordance with our results, Russel and Hugo [24] and Narayanan *et al*. [25] reported the biocidal effect of silver-based compounds against wide range of bacteria such as *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*, *Salmonella* Typhi and *Klebsiella pneumonia*.

Table 3: Antibacterial activity of neomycin alone and in combination with silver nanoparticles.

Microorganisms	MIC			FICI	Effect
	Neomycin alone	Combination			
	(mg/mL)	(µg/mL)	(mg/mL)		
***Escherichia coli* O1**	64	0.2125	8	0.375	Synergy (S)
***Escherichia coli* O2**	32	0.2125	8	0.5	Synergy (S)
***Escherichia coli* O78**	32	1.7	64	4	Antagonism
***Salmonella* Typhimurium**	16	1.7	64	6	Antagonism
***Salmonella* Enteritidis**	64	0.85	32	1.5	Indifferent

S: Synergistic. A: Additive. I: Indifferent.

This action of bactericidal capability of AgNPs was attributed to several mechanisms; firstly, their small size and large dispersion surface to volume ratio which allows them to interact closely with microbial membranes [26,27]. Thus, helping the Silver nanoparticles (AgNPs) to combine with the bacterial cell wall and membrane and leads to stopping the respiration progression [28]. These effects lead to breakdown of the plasma membrane causing exhaustion of intracellular ATP [29]. Secondly, the metal oxides carry the positive charge while the microorganism carry negative charge, that causes electromagnetic attraction between microorganism and the metal oxides and leads to oxidization and finally death of microorganism [30,31]. Finally, nano-Ags generate hydroxyl radicals, a highly reactive oxygen species induced by bactericidal agents [32].

Our findings showed the bactericidal effects of silver nanoparticles that synthesized by Rosemary on *E. coli* and *Salmonella* strains as well as a synergistic effect with gentamycin. This result was in agreement with Lee *et al.* [33]; Markowska *et al.* [34]; Singh *et al.* [35] who reported preliminary results concerning the synergistic effect of amoxicillin and AgNPs against *E. coli* and no synergetic effect was emphasized in combinations of AgNPs with oxacillin, ciprofloxacin, meropenem, and ceftazidime.

In a previous study on the synergistic effect of nanoparticles with gentamicin and neomycin on *S. aureus* causing mastitis, Jamaran and Zarif [36] reported the synergistic effects of gentamicin with AgNPs on 50% of the examined strains and on 45% of the strains when the neomycin was used with AgNPs. In the same respect, Birla *et al.* [37] observed the synergistic action between silver nanoparticles (AgNPs) and ampicillin, gentamicin, kanamycin, streptomycin and vancomycin against *E. coli*.

The current study also clearly demonstrated that antibiotic-resistant bacteria become susceptible when an antibiotic was combined with AgNPs as proved in case of the synergistic effect of ampicillin with AgNPs against *E. coli*. This phenomenon was reported previously by Singh and Brown [35,38]. In addition, Brown *et al.* [38] reported that AgNPs with ampicillin were effective against ampicillin-resistant *E. coli*.

Conclusion

A strong synergistic effect of broad-spectrum antibiotics with well characterized AgNPs silver nanoparticles of an average size of 8.5 nm to 16.5 nm synthesized using rosemary extract was observed. This green synthesis method is eco-friendly than chemical method. It is also inexpensive and pollutant free. Our findings support the argue that nano-Ags have substantial effective antibacterial

activity and merit further investigation for field trials in poultry farms.

Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

تحسين الحساسية للمضادات الحيوية لميكروبي السالمونيلا والايشريشياكولاي المعزولة من الدجاج باستخدام جزيئات النانو للفضة في محافظة أسيوط

مؤمن عبدالعظيم محمد[١]، فاطمة مختار محمد[٢]، وليد أحمد السيد[٣]

[١]قسم أمراض الدواجن، كلية الطب البيطرى، جامعة أسيوط-أسيوط
[٢]قسم أمراض الدواجن، معهد بحوث صحة الحيوان، المعمل الأقليمى أسيوط-أسيوط
[٣]قسم الكيمياء،كلية العلوم، جامعة أسيوط-أسيوط

قبل أن ندخل عصر المقاومة الكاملة للمضادات الحيوية وذللك للاستخدام الشائع للمضادات الحيوية، تم اكتشاف استراتيجيات جديدة، واحدة منها استخدام الجسيمات النانوية أو متناهية الصغر لتعزيز عمل المضادات الحيوية. لذلك، تم تنفيذ هذه الدراسة لمعرفة التأثير المضاد لجسيمات الفضة متناهية الصغر على االبكتريا إما بشكل منفصل أو بالاتحاد مع المضادات الحيوية المستخدمة. وأظهرت النتائج التي تم الحصول عليها للنشاط المضاد للبكتيريا لجسيمات الفضة متناهية الصغر ضد عترات الايكولاي والسالمونيلا عند مستوى٨٥.٠ ميكروجرام / مل. كما أظهرت نتائجنا تأثيرات متآزرة مختلفة للمضادات الحيوية المستعملة على عترات الايكولاي المستهدفة حيث كان التأثير المثبط الأدنى للمضادات الحيوية المستخدمة مع جسيمات الفضة متناهية الصغر في الغالب مرتين أقل من تلك المضادات الحيوية المستخدمة بمفردها (سيبروفلوكساسين وأموكسيسيلين) وأكثر من ثلاثة أضعاف مع جنتاميسين، سيفوتاكسيم والنيومايسين. ولوحظ نتيجة مدهشه في حالة تأثير التآزر أموكسيسيلين و جنتاميسين مع جسيمات الفضة متناهية الصغر حيث أظهر استخدام هذه المضادات الحيوية وحدها ضد الايكولاي-O2 مقاومة لهذه المضادات الحيوية، ولكن عند إضافة جسيمات الفضة متناهية الصغر إلى أموكسيكسيلين و جنتاميسين تم استعادة النشاط مضاد للجراثيم لهذه المضادات الحيوية. على النقيض لوحظ تأثير جسيمات الفضة متناهية الصغر مع المضادات الحيوية على عترات السالمونيلا المسخدمة كالتالى، كان يترواح بين عديم التأثير والمتعارض. وأخيرا، يمكن أن نخلص إلى أن جسيمات الفضة متناهية الصغر يمكن أن تنتج بسهولة من قبل المستخلصات المائية للروزماري وهي منخفضة التكلفة وطريقة صديقة للبيئة. وأيضا أن استخدام جسيمات الفضة متناهية الصغر كان مشجعا ضد عترات ال الايكولاي إما وحدها أو في خلطها من المضادات الحيوية التي تم اختبارها حيث من الممكن ان تكون مناسبة فى تكوين اجيال جديدة من المركبات القاتلة للبكتريا وأمكان استخدامها فى مزارع الدواجن لمنع وعلاج عدوى الايكولاى.