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Minimum bactericidal concentration of chemically synthesized silver nanoparticles against pathogenic *Salmonella* and *Shigella* strains isolated from layer poultry farms

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ABSTRACT

The main objective of the present study is to find an appropriate *in-vitro* therapy to overcome clinically isolated antibiotic-resistant *Salmonella* and *Shigella* species from cases of layer poultry farms suffering from purulent dysentery and diarrhea. The present study demonstrated chemical synthesis of silver nanoparticles (AgNPs) via chemical reduction method and investigation for their antibacterial effect against the isolated bacteria by determining their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Of 32 examined samples, 8 (25%) antibiotic-resistant isolates were isolated and identified including: *Shigella flexneri*, *Salmonella typhimurium* (two), *Salmonella poona*, *Shigella boydii*, *Salmonella montevideo*, *Shigella sonnei*, *Salmonella enteritidis*. Spherical AgNPs of 10-25 nm in size were synthesized and the AgNPs at a concentration of 16 μgml^{-1} were found to have both bacteriostatic and bactericidal effects in the case of *Salmonella montevideo* (layer chicken egg), *Shigella sonnei* (layer chicken feces), *Salmonella enteritidis* (layer duck egg) however the AgNPs at a concentration of 8 μgml^{-1} were found to have both bacteriostatic and bactericidal effects in the case of *Salmonella poona* (layer chicken feces), *Shigella boydii* (layer chicken feces), *Salmonella typhimurium* (layer chicken feces).

INTRODUCTION

Salmonella and *Shigella* species are Gram-negative rod-shaped bacteria that are members of the family Enterobacteriaceae and are considered threatened foodborne pathogens facing food safety and public health (Arena *et al.*, 2017; Pashazadeh *et al.*, 2017). Depending on serology, *Salmonella* species comprises over 2500 serotypes (Pashazadeh *et al.*, 2017) while *Shigella* comprises four subspecies: *Shigella dysenteriae* (serogroup A, 15 serotypes), *Shigella flexneri* (serogroup B, 14 serotypes), *Shigella boydii* (serogroup C, 20 serotypes), and *Shigella sonnei* (serogroup D, a single serotype) (Anderson *et al.*, 2016; Arena *et al.*, 2017). *Salmonella* is a serious threat facing poultry industries as it has the ability to infect chickens causing diarrhea (Hsu *et al.*, 2016). In the last few years, consumption of contaminated poultry, eggs, and their products become the most common sources of foodborne human salmonellosis (Mahmoud *et al.*, 2015; Park *et al.*, 2015; Kalupahana *et al.*, 2017). On the other hand, Shigella has the ability to infect chickens, which consequently could infect human causing shigellosis (Xu *et al.*, 2004; Shi *et al.*, 2014). The applications of antibiotics for treatment of bacterial infections or as growth promoters have a bad side effect, including: persistence of antibiotics residues in animal products as meat and eggs (Namagirilakshmi *et al.*, 2010; Hsu *et al.*, 2016), another bad side

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effect is the high incidence of an antibiotic-resistant bacterial species development which is considered one of the public health hazards (Tseng *et al.,* 2014; Hsu *et al.,* 2016). Therefore, it is important to find another efficient treatment for *Salmonella* and *Shigella* infection instead of antibiotic. In the last few years, there has been a growing interest in nanotechnology. Indeed, nanoparticles have been gaining importance in recent years and became an effective revolution therapy against pathogenic bacteria due to their bactericidal properties. The nanoparticles size and surface area are significant agents to which their bactericidal mechanism of action attributed to (Devi *et al.,* 2017). Silver nanoparticles (AgNPs) has been gaining importance in recent years due to their broad spectrum bactericidal toxic effects against broad spectrum pathogenic bacteria (Ahmad *et al.,* 2017) even at their minimum concentrations (Devi *et al.,* 2017). Furthermore, the results offered by Raman *et al.,* 2017 suggest that AgNPs can be used as potent bactericidal agents against antibiotic-resistant bacteria. However, to the best of our knowledge, most of the previous studies did not take into account AgNPs bactericidal effect against pathogenic antibiotic-resistant *Salmonella* and *Shigella* strains clinically isolated from layer poultry farms. The goal of the present study is to clinically isolate and identify *Salmonella* and *Shigella* strains from cases of layer poultry (chickens and ducks) farms suffering from purulent dysentery and diarrhea. The present work was also intended to chemically synthesize AgNPs via chemical reduction method. The synthesized AgNPs will be characterized through high-resolution transmission electron microscopy (HRTEM), ultraviolet–visible (UV–vis) spectroscopy, and Malvern-Zetasizer Nano (ZSP) – UK. The antibacterial effect of the synthesized AgNPs will be investigated through applying of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the clinically isolated *Salmonella* and *Shigella strains*.

MATERIALS AND METHODS

Samples collection

A total of 32 samples (15 layer chicken feces, 5 layer chicken eggs, 9 layer duck feces, 3 layer duck eggs) were randomly collected from cases of layer poultry farms suffering from purulent dysentery and diarrhea. The samples were collected aseptically under a sterile condition and transported in an insulated ice box within 4 hours of the collection to the Microbiology and Immunology Department, Veterinary Division, National Research Centre, Cairo, Egypt, where they were analyzed immediately for the presence of pathogenic *Salmonella* and *Shigella*.

Salmonella and *Shigella* Isolation and Identification

For the isolation of *Salmonella* from chicken or duck feces, a sterile swab of ≈1g fecal sample was collected and added to 50 ml buffered peptone water (Oxoid), blended for 2 min, left for 60 min at room temperature and then incubated at 37°C for 18 hours. After incubation, 0.1 and 1 ml from the previous broth were transferred to 10 ml Rappaport Vassiliadis (RV) (Oxoid) and 10 ml tetrathionate (TT) (Oxoid) broth respectively, followed by 24 h incubation at 42°C and 35°C for RV medium and TT broth respectively. After incubation, a loopful from each broth was streaked onto 2 agar medium (Oxoid) including: Xylose lysine deoxycholate (XLD) and *Salmonella-Shigella* (SS) plates and then incubated both at 37°C for 24 hours. On the other hand, for the isolation of *Shigella* from chicken or duck feces, the fecal sample was collected using a sterile swab in sterile tube containing 5 ml buffered peptone water, and then incubated at 37°C for 24 hours and then a loopful from each tube was inoculated onto SS agar medium plates and then incubated at 37°C for 24 hours. Meanwhile, for the isolation of *Salmonella* and *Shigella* from chicken or duck eggs, the whole eggs were washed with soap and then rinsed in 70% ethanol for 20 min for surface sterilization. 1ml of the separated egg yolk was added to 50 ml lactose broth and 5 ml buffered peptone water for the isolation of *Salmonella* and *Shigella* respectively and then completed as mentioned before. After incubation of all cultured petri-dishes, the suspected colonies were then examined through biochemical identification methods followed by API system (Biomerieux). Serological identifications were done using *Salmonella* and *Shigella*-specific antisera. All identified isolates were then stored at -20°C in brain heart infusion broth (Oxoid) supplemented with 15% (v/v) sterile glycerol.

Antibiotic susceptibility of the isolated *Salmonella* and *Shigella* strains

The antimicrobial sensitivity of the isolated *Salmonella* and *Shigella* was performed by the Kirby-Bauer disk diffusion method according to guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2013). Mueller Hinton agar medium (MH, Oxoid) was used. The antibiotic discs (Oxoid) used, including: Norfloxacin (10 µg), Amoxiclav (Amoxicillin/ Clavulanic acid) (30 µg), Tobramycin (10 µg), Oxytetracycline (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Gentamicin (10 µg), Ampicillin (10 µg), and Tetracycline (30 µg). Multidrug-Resistant (MDR) strains were also determined. Any isolate resistant to ≥ 3 is defined as multidrug-resistant pathogen (Magiorakos *et al.,* 2012; Omara *et al.,* 2014).

Materials used for silver nanoparticles synthesis

All chemicals and reagents used for silver nanoparticles (AgNPs) synthesis were of analytical grades. Silver nitrate (≥ 99.9%), sodium borohydride (≥ 98%), tri-sodium citrate dihydrate (≥ 99%), hydrogen peroxide (50%), and polyvinylpyrrolidone were purchased from Sigma-Aldrich and used as received without further purification. All solutions were prepared using de-ionized water. The borohydride solution was freshly prepared.

Preparation and characterization of silver nanoparticles

AgNPs sample was synthesized by the chemical reduction method. It was prepared by adding 3.5 ml sodium citrate (30 mM), 3.5 ml PVP, and 45 µl H_2O_2 into the freshly prepared $AgNO_3$ (50 ml, 0.50 mM in deionized water) under vigorous stirring. After a few minutes, 250 µl of 80 mM $NaBH_4$ was added

to obtain colloidal AgNPs. This mixture was stirred for 3 hours and stored in dark at 4 ^{o}C until use (Métraux and Mirkin, 2005). The size and shape of the prepared AgNPs colloidal solution were investigated using HRTEM (a JEOL JEM- 1200 EX high-resolution transmission electron microscope) operating at 120 kV. UV-visible spectrum of the synthesized AgNPs suspension was obtained on a JASCO UV-VIS spectrophotometer. The particle size distribution of the prepared AgNPs was measured by Malvern-Zetasizer Nano (ZSP) - UK; Size measurement from 0.3 nm (diameter) to 10 microns.

Silver nanoparticles preparations for the MIC and MBC experiment

A stock solution of AgNPs 32 µg/ml (size range of 10-25 nm) was prepared, then two-fold serial dilutions were done using Mueller Hinton broth medium (MH broth, Oxoid) to obtain the following concentrations 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 and 0.063 µg/ml.

Microorganisms used for evaluation of Antibacterial activities of AgNPs

Stock cultures of the clinically isolated and identified eight pathogenic antibiotic-resistant *Salmonella* and *Shigella* strains in the present study including: *Shigella flexneri* (layer chicken feces), *Salmonella typhimurium* (layer duck feces), *Salmonella poona* (layer chicken feces), *Shigella boydii* (layer chicken feces), *Salmonella typhimurium* (layer chicken feces), *Salmonella montevideo* (layer chicken egg), *Shigella sonnei* (layer chicken feces), *Salmonella enteritidis* (layer duck egg) were used for evaluation of the antibacterial activities of the synthesized AgNPs. Test organisms were first activated from glycerol by two successive transfers in brain heart infusion broth at 37°C for 24 hours, then streaking on XLD and SS medium for *Salmonella* and *Shigella*, respectively. A single pure colony was streaked on brain heart infusion slants then incubated at 37°C for 24 hours, followed by storing at 4°C until the beginning of the experiment.

Bacterium Inoculum preparations for the MIC and MBC experiment

Loopful of culture from each previously inoculated brain heart infusion slants was transferred into brain heart infusion broth. The broth was then incubated with shaking at 37°C for overnight. Then the concentrations of these suspensions were adjusted to be equal to 5×10^5 CFU/ml using sterile saline (Omara, 2017).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the chemically synthesized AgNPs against the isolated *Salmonella* and *Shigella* strains

Antimicrobial activities of the chemically synthesized AgNPs against the clinically isolated antibiotic-resistant *Salmonella* and *Shigella* isolates were analyzed through determination of the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and MBC/MIC ratio values using a broth microdilution method in the 96-well micro-titer plates (Omara, 2017). To each well, 50 µl of the adjusted bacterial inoculum (5×10^5 CFU/ml) were added horizontally, while 50 µl of the AgNPs dilution were added vertically. Each plate included growth and sterility control wells; the growth control wells contained MH broth medium with tested bacterial concentrations in order to check the bacterial viability, while the sterility control wells contained only a sterile MH broth in order to check the sterility of the medium used.

The plates were covered slackly with cling film to ensure that the bacteria were not dehydrated and then incubated at 37°C for 18-20 hours. The lowest concentration of each antibacterial agent that inhibited the bacterial growth was then considered as the MIC (CLSI, 2013). After MIC determination, aliquots of 100 µl from each well, which showed no bacterial growth after incubation was seeded in BHI agar plates, which are not supplemented with AgNPs, then were incubated at 37 °C for 20 hours. The lowest concentration of the AgNPs that kills 100% of the initial bacterial population showing no colony on the MH agar after 20 hours of incubation at 37°C was recorded as the MBC (CLSI, 2013; Omara, 2017).

RESULTS

Isolation and identification of *Salmonella* and *Shigella*

Out of 32 examined samples, 8 (25%) strains were isolated and identified including: *Shigella flexneri (S. flexneri)* (layer chicken feces), *Salmonella typhimurium (S. typhimurium)* (layer duck feces), *Salmonella poona* (*S. poona*) (layer chicken feces), *Shigella boydii* (S. boydii) (layer chicken feces), *Salmonella typhimurium (S. typhimurium)* (layer chicken feces), *Salmonella montevideo (S. montevideo*) (layer chicken egg), *Shigella sonnei* (*S. sonnei*) (layer chicken feces), *Salmonella enteritidis* (*S. enteritidis*) (layer duck egg).

Antimicrobial sensitivity of the isolated *Salmonella* and *Shigella*

Table 1 summarizes the results of antibiotic sensitivity of the isolated *Salmonella* and *Shigella*, it can be seen that all the isolated *Salmonella* and *Shigella* were multidrug resistant pathogens.

Table 1: Antimicrobial susceptibility of the isolated *Salmonella* and *Shigella* strains (n = 8).

Antimicrobials	Antimicrobial susceptibilities					
	Sensitive		Intermediate resistant		Resistant	
	No	%	No	%	No	%
Norfloxacin	3	37.5	-	-	5	62.5
Amoxiclav	1	12.5	1	12.5	6	75
Tobramycin	-	-	-	-	8	100
Oxytetracycline	-	-	-	-	8	100
Ciprofloxacin	2	25	1	12.5	5	62.5
Imipenem	-	-	-	-	8	100
Gentamicin	-	-	-	-	8	100
Ampicillin	1	12.5	2	25	5	62.5
Tetracycline	-	-	-	-	8	100

Characteristics of silver nanoparticles

Figure 1 shows high-resolution transmission electron microscopy (HRTEM) image of the synthesized AgNPs. Well homogenous shape of AgNPs is obtained; most of the particles were spherical but some of them having prismatic shape and due to their higher reactivity, the particles tend to agglomerate. Moreover, the size range of the obtained AgNPs are between 10-25 nm. The result of the particle size distribution of the synthesized AgNPs is presented in Fig. 2 which confirms the result obtained from HRTEM analysis.

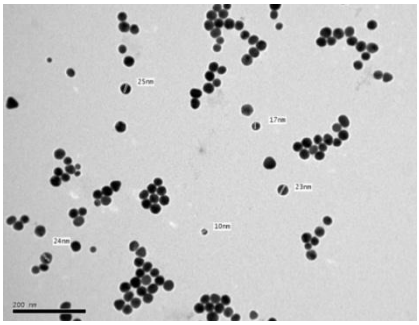

Fig. 1: HRTEM image of the synthesized AgNPs.

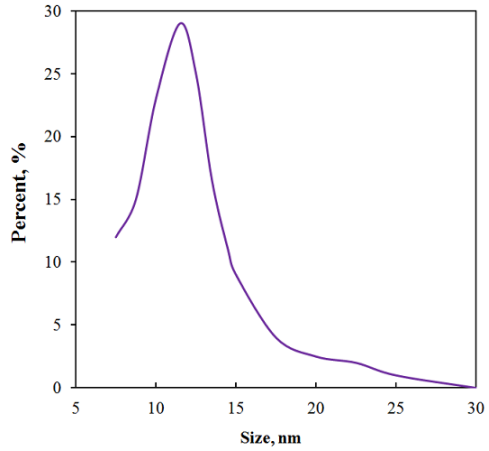

Fig. 2: Particle size distribution of the synthesized AgNPs.

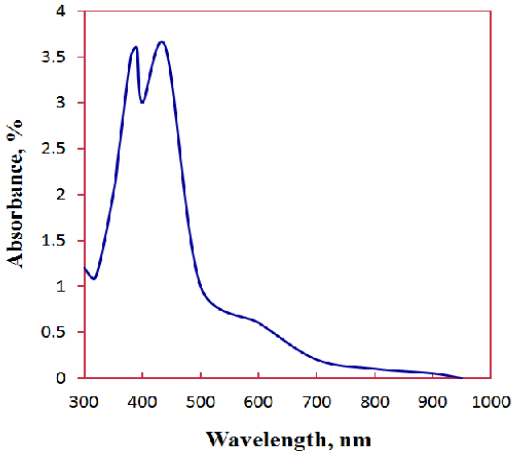

Fig. 3: UV-Vis. spectrum of the synthesized AgNPs.

To investigate the structural characteristics of AgNPs, UV-vis spectroscopy technique was used. Fig. 3 depicts Uv-Vis. spectrum of synthesized AgNPs. The spectrum exhibits two absorption shoulders at wavelength ranging between 350 and 450 nm, indicating the presence of spherical and roughly spherical Ag nanoparticles.

MIC and MBC determination of chemically synthesized AgNPs against the isolated *Salmonella* and *Shigella strains*

The MIC (µgml^{-1}) and MBC (µgml^{-1}) values as well as MBC/MIC ratios of the prepared AgNPs against the eight isolated Salmonella and Shigella strains are presented in Figure 4 and illustrated in Table 2.

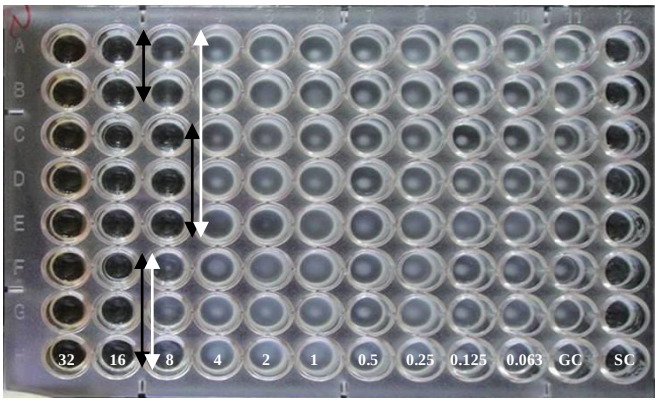

Fig. 4: Microtiter plates showing the MICs and MBC of AgNPs. Rows A–H: *Shigella flexneri* (layer chicken feces), *Salmonella typhimurium* (layer duck feces), *Salmonella poona* (layer chicken feces), *Shigella boydii* (layer chicken feces), *Salmonella typhimurium* (layer chicken feces), *Salmonella montevideo* (layer chicken egg), *Shigella sonnei* (layer chicken feces), *Salmonella enteritidis* (layer duck egg) respectively. Columns 1–10: contain twofold serial dilutions of 32 µgml-1 AgNPs, columns 11: GC (growth control wells), columns 12: SC (sterility control wells). White and black arrows indicate the MICs and MBCs respectively against each isolate.

Table 2: MIC (µgml^{-1}) and MBC (µgml^{-1}) values as well as the MBC / MIC ratio of AgNPs against the isolated *Salmonella* and *Shigella* species.

Rows	Bacterial isolates	Antimicrobial activity of AgNPs		
		MIC (µgml^{-1})	MBC (µgml^{-1})	MBC/MIC ratio
A	*Shigella flexneri* (layer chicken feces)	8	16	2
B	*Salmonella typhimurium* (layer duck feces)	8	16	2
C	*Salmonella poona* (layer chicken feces)	8	8	1
D	*Shigella boydii* (layer chicken feces)	8	8	1
E	*Salmonella typhimurium* (layer chicken feces)	8	8	1
F	*Salmonella montevideo* (layer chicken egg)	16	16	1
G	*Shigella sonnei* (layer chicken feces)	16	16	1
H	*Salmonella enteritidis* (layer duck egg)	16	16	1

DISCUSSION

Annually, 155000 death cases were reported due to *Salmonella* foodborne gastroenteritis (Liu *et al.*, 2013). Recently, a total of 356 persons from 39 US states were infected by *S. typhimurium* in an outbreak, which occurred due to contact with backyard chicks and ducklings (CDC, 2013; Zheng *et al.*, 2014). In the present study, 8 (25%) Salmonella and Shigella strains were isolated and identified out of the examined 32 samples. Previously,

a total of 127 *Salmonella* isolates were isolated from fecal samples of chicken suffering from diarrhea (Kang *et al.,* 2017). On the other hand, *Shigella* has been isolated from cases of chicken shigellosis suffered from bloody and purulent dysentery (Xu *et al.,* 2004; Shi *et al.,* 2014). Moreover, *Shigella* species were detected in 2 (0.25%) of previously examined chicken samples (Ahmed and Shimamoto, 2014). Isolation of *Salmonella* serovars from ducks has been also recorded from many countries including Egypt (Osman *et al.,* 2014; Tang *et al.,* 2015). In addition, previously examined raw duck meat has been shown to be a reservoir of *Salmonella* species, especially *S. typhimurium* (Adzitey *et al.,* 2012). Furthermore, out of total collected 531 duck samples from duck farms, 125/531 (23.5%) were positive for *Salmonella* infection (Adzitey *et al.,* 2012). On the other hands, *Salmonella* can reach also the egg yolk by either vertical transmission or horizontal transmission or even through temperature. If *Salmonella* reaches the egg yolk, it can then grow rapidly, even at room temperature (25°C) (Martelli and Davies, 2012). The vertical transmission (trans-ovarian infection) can be contributed to colonization of *Salmonella* to the layer reproductive organs (Kalupahana *et al.,* 2017) in which *Salmonella* can then pass easily to the yolk, yolk membranes, and albumen of the newly formed eggs (Park et al., 2015). While, the horizontal transmission can be contributed to the contact between eggs and anything contaminated with *Salmonella* as handlers hand, layer fecal matter, pests, and even utensils in which, *Salmonella* can then pass easily through the shell membrane to the egg contents (Park *et al.,* 2015). Furthermore, the positive differential temperature creates a negative pressure in which, if there is a moist environment at the shell surface, *Salmonella* can then pass easily to the egg contents (Martelli and Davies, 2012).

Recently, multidrug-resistant (MDR) bacteria have been spread worldwide which represent serious threat public health hazards. In the present study, all the isolated *Salmonella* and *Shigella* strains were multidrug resistance pathogens as summarized in Table 1. The administration of antibiotic for treatment of *Salmonella* or as growth promoters resulted in development of varying degree of *Salmonella* resistant strains as recorded by Carrique-Mas *et al*. (2008), Osman *et al*. (2014); and Tang *et al*. (2015). A multidrug-resistant (MDR) *Salmonella* have been previously recorded (Tsai and Hsiang, 2005; Cha *et al*., 2013; Chang *et al*., 2014; Tang *et al*., 2015).

This study presents a pilot study to answer how to overcome antibiotic-resistant *Salmonella* and *Shigella*. In the present study, chemically synthesized silver nanoparticles (AgNPs) by reduction method showed efficient antibacterial activities against clinically isolated antibiotic-resistant *Salmonella* and *Shigella* strains. In a previous study, a broad spectrum antibacterial activity of AgNPs against various pathogenic strains of bacteria, including *Salmonella* (Boonkaew *et al.,* 2014; Patil *et al.,* 2015) specially *S. typhimurium* were reported (Abd-Elnaby *et al.,* 2016) similar to our results. Previously, silver nanoparticles (AgNPs) were reported as an excellent bactericidal agent used for killing bacteria (Raman *et al.,* 2017). The nanoparticles bactericidal properties are attributed mainly to the electrostatic reaction between negative charge of bacterial cell wall and positive charge of nanoparticles metal ions (Prema *et al.,* 2017). The shape, size, surface area, and stability of AgNPs are considered the main factors affecting on AgNPs bactericidal properties (Gliga *et al.,* 2014; Su *et al.,* 2017a). In the present study, the prepared AgNPs are spherical, and a previous study reported that the spherical AgNPs were found to have wider antibacterial activity in Gram-negative organisms (Swarnavalli *et al.,* 2017). In the present study, UV-vis spectroscopy technique used to investigate the structural characteristics of the synthesized AgNPs as presented in Fig. 3, the spectrum exhibits two absorption shoulders at wavelength ranging between 350 and 450 nm, indicating the presence of spherical and roughly spherical Ag nanoparticles. Moreover, when the peak intensity is increased, this means the formation of spherical seed particles. It is important to mention that smaller particles give sharp peaks at shorter wavelengths less than 400 nm as compared to large particles which give broad peaks at longer wavelengths more than 400 nm. Previous study reported that when the absorption peak is ≤400 nm, the size range of nanoparticles would be 10-15 nm and when it is 400-440 nm the corresponding sizes are 20-60nm (Abideen, 2012). The smaller size NPs and larger surface area play a vital role in the antimicrobial performance of nanoparticles (Zafar *et al.,* 2016; Su *et al.,* 2017a). The smaller sized AgNPs (10-25nm) obtained in the present study is considered as an enhancing factor responsible for that observable antibacterial activity against tested *Salmonella* and *Shigella*. The results obtained in the present study are consistent with that of other research articles. Indeed, smaller sized AgNPs demonstrated higher bactericidal effect against pathogenic and non-pathogenic bacteria than their larger sized particles (Swarnavalli *et al.,* 2017; Su *et al.,* 2017a). Moreover, the bactericidal properties of AgNPs improved by decreasing AgNPs´ size (Su *et al.,* 2017a). This phenomenon can be explained by the following; when size of AgNPs decreases, the maximum contact area will increase, and the surface area to volume ratio (SA/ V) for each AgNP will then increase, and this resulting in increasing in relative AgNPs concentration (Agnihotri *et al.,* 2014; Swarnavalli *et al.,* 2017). This can be attributed to higher particle penetration and availability of more area of contact between the bacteria and nanoparticles (Swarnavalli *et al.,* 2017).

Furthermore, the bactericidal activities of AgNPs against Gram-negative bacteria depend on the concentration of AgNPs (Sondi and Salopek-Sondi, 2004; Swarnavalli *et al.,* 2017). Previously, Swarnavalli *et al.,* 2017 reported that, with increasing the concentration of AgNPs, the higher antibacterial effects were observed (as done when the concentration of AgNPs increased from 0.49 to 250 μgml^{-1}). In the present study, it is clear that the MIC and MBC values of AgNPs against the tested bacterial isolates were either 8 or 16 μgml^{-1}. The MBC of AgNPs was observed mainly at a concentration of 16 μgml^{-1} AgNPs in the case of *Shigella flexneri* (layer chicken feces), *Salmonella typhimurium* (layer duck feces), *Salmonella montevideo* (layer chicken egg), *Shigella sonnei* (layer chicken feces), *Salmonella enteritidis* (layer

duck egg) while it was 8 μgml^{-1} AgNPs in the case of *Salmonella poona* (layer chicken feces), *Shigella boydii* (layer chicken feces), *Salmonella typhimurium* (layer chicken feces).

On the other hand, from the MIC and MBC results obtained in the present study, it is clear that, the AgNPs at a concentration of 16 μg ml^{-1} was found to have both bacteriostatic and bactericidal effects, followed by a concentration of 8 μg ml^{-1}. In previous studies, the MIC of a chemically synthesized nanoparticles (30–40 nm) against *Salmonella* were reported at 2.81 μgml^{-1}. Moreover, the efficient bactericidal activity of AgNPs was obtained at its lowest concentration of 0.35 μgml^{-1} (Devi *et al.*, 2017). Furthermore, the MBC value of 100 nm sized AgNPs was ≈1 × 104 μgml^{-1}, while that for 5–10 nm sized AgNPs was 12–25 μgml^{-1} (Flores *et al.*, 2013). A previous study reported that the calculated MIC of 28 nm sized AgNPs against *Salmonella* was 25 μgml^{-1} while it was 12.5 μgml^{-1} in case of 8 nm sized AgNP (Smekalova *et al.*, 2016). Furthermore, the 3.5 μgml^{-1} MIC and 6.9 μgml^{-1} MBC values of AgNPs against *Salmonella enterica* [ATCC 13076] were previously reported (Singh *et al.*, 2016). Similar to the present study, a chemically synthesized AgNP sized 30–40 nm showed MIC against *Salmonella* at a concentration of 2.81 μgml^{-1} (Zafar *et al.*, 2016).

The AgNPs' bactericidal properties are attributed mainly to their following abilities: bacterial cell walls and membrane destruction, reactive oxygen species (ROS) generation, vital metabolic enzyme inhibition, and obstruction of DNA replication (Feng *et al.*, 2000; Sondi and Salopek-Sondi, 2004; Kim *et al.*, 2007; Rai *et al.*, 2009; Ahmad *et al.*, 2017). AgNPs have the ability to attach and destruct bacterial cell walls in a dose-dependent manner (Cerny and Teuber, 1971; Rhayour *et al.*, 2003; Ahmad *et al.*, 2017) this is confirmed by Electron spin resonance spectroscopy studies which demonstrated that when AgNPs attached to the bacterial cell walls, the free radicals of the AgNPs were then released (Danilcauk *et al.*, 2006; Kim *et al.*, 2007; Swarnavalli *et al.*, 2017).

These released free radical seems to be the main causative agent responsible for final bacterial destruction and death (Devi *et al.*, 2017); as these free radicals begin to make pores in the bacterial cell walls and membranes causing severe permeability of the bacterial cell membrane (Danilcauk *et al.*, 2006; Kim *et al.*, 2007; Rai *et al.*, 2017; Swarnavalli *et al.*, 2017), this is followed by releasing of periplasmic proteins and nucleic acids via cytoplasmic membrane, and so the bacterial cell death will be the final result (Cerny and Teuber, 1971; Rhayour *et al.*, 2003; Ahmad *et al.*, 2017; Rai *et al.*, 2017; Swarnavalli *et al.*, 2017). On the other hand, when AgNPs enter the bacterial cell, they form a low molecular weight region which begin to attack the respiratory chain, so consequently, the cellular signaling pathways will be changed by dephosphorylating assumed key peptide substrates on tyrosine residues (Sondi and Salopek-Sondi, 2004; Mulley *et al.*, 2014; Su *et al.*, 2017b). Moreover, the Ag+ metal ions that released from AgNPs (Feng *et al.*, 2000; Swarnavalli *et al.*, 2017) were found to have obstructing behavior to the bacterial signal transduction pathways (Devi *et al.*, 2017) and moreover they could react with thiol groups present in the bacterial vital enzymes and proteins (Matsumura *et al.*, 2003; Swarnavalli *et al.*, 2017; Su *et al.*, 2017b; Gopinath *et al.*, 2016) and furthermore, they could react with phosphorus-containing compounds like DNA leading to inhibition of bacterial DNA replication, and so the bacterial cell death will be the final result (Jiao *et al.*, 2014; Bao *et al.*, 2015; Su *et al.*, 2017b; Devi *et al.*, 2017). On the other hand, the production of intracellular reactive oxygen species (ROS) by AgNPs occurred via the reaction between the AgNPs with the thiol-containing enzymes of the bacterial respiratory chain (Matsumura *et al.*, 2003; Ahmad *et al.*, 2017). The ROS seems to another causative agent responsible for final bacterial destruction and death (Shrifian-Esfahni *et al.*, 2015; Ahmad *et al.*, 2017) as this ROS is cytotoxic fatal to the bacterial cell in dose and surface charge dependent manner (Ahmad *et al.*, 2017). Indeed, the formed ROS generates an oxidative stress to the bacterial cell resulting in suppression of the bacterial antioxidant defense mechanism, destruction of the bacterial cell membrane as well as DNA and vital enzymes, these circumstances resulting in final bacteria cell death (Kim *et al.*, 2007; Li *et al.*, 2010; Reuter *et al.*, 2010; Ahmad *et al.*, 2017).

CONCLUSION

From the outcome of our investigation, this study demonstrated high level (25%) of antibiotic-resistant *Salmonella* and *Shigella* infection, out of 32 examined samples of layer chickens and ducks as well as their eggs. This study suggesting possible applications of chemically synthesized spherical silver nanoparticles (10-25 nm) as an efficient bactericidal agent against clinically isolated antibiotic-resistant *Salmonella* and *Shigella* strains. The MBC of AgNPs was best observed mainly at a concentration of 16 μgml-1 followed by 8 μgml-1. In-vivo clinical applications of AgNPs to verify their *in-vivo* effectiveness will be the next step of our research studies.

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