Egyptian Journal of Animal Health

P-ISSN: 2735-4938 On Line-ISSN: 2735-4946
Journal homepage: https://ejah.journals.ekb.eg/

Antibacterial Effect of Silver Nanoparticles Biosynthesized Using *Aspergillus niger* on *E.coli* Isolated From Chicken

Flourage M. Rady [*], Eman. F.E [**] and Gamal A.M Omran [***]

[*] Mycology Lab, [**] Poultry Diseases Lab., [***] Bacteriology Lab., Animal Health Research Institute (AHRI), Shbeen El Koom Lab, Agriculture Research Center (ARC)

Received in 16/8/2023
Received in revised from 3/9/2023
Accepted in 20/9/2023

Keywords:

Silver nanoparticles
E.coli
Aspergillus niger
Chicken

ABSTRACT

E.coli is believed to be one of the most main bacteria in the population which constitutes the normal microbial flora. *E.coli* strains have been found in the gastrointestinal tract of humans, poultry and other animals as harmless bacteria, but despite the harmless nature of its existence, *E.coli* can be opportunistic if the host immunity status wasn't intact. In this research work, evaluation, and investigation of the anti-bacterial activity of silver nanoparticles which synthesized by a green chemistry methodology through utilizing *Aspergillus niger* was carried out. The isolation of *E.coli* bacteria has been completed through sampling of different regions of the chickens, such as the cloacal region, intestinal fluid, and faecal material. For identification of *E.coli,* biochemical examinations, like catalase, oxidase, urease, triple sugar iron test, citrate utilization test, and indole test were applied. Silver nanoparticles characterization was accomplished through using different physicochemical techniques, namely, Fourier transformed infrared (FT-IR) which has been used to obtain the distinctive molecular fingerprint of silver nanoparticles through the formation of the infrared absorption spectrum, Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) to display the morphology of the nanoparticles. Agar well diffusion method has been executed to evaluate how effective silver nanoparticles are as anti-bacterial agent against *E.coli* strains by means of inhibition zone and MIC values. The values of the inhibition zone and MIC obtained were 14 mm and 39.06 µg/ml respectively.

INTRODUCTION

E. coli is deemed as one of the common microbial flora of the gastrointestinal tract of poultry. It could be either non-pathogenic or pathogenic causing systemic disease in poultry (avian colibacillosis). This is an infectious disease leading to acute fatal septicemia or subacute fibrinous pericarditis, airsacculitis, peri-

Corresponding Author: Flourage M. Rady, Mycology Lab, Animal Health Research Institute (AHRI), Shbeen El Koom Lab, Agriculture Research Center (ARC)
E-mail: flouragerady206@gmail.com
DOI: 10.21608/ejah.2023.322761

tonitis, and salpingitis in 4–6 weeks aged broiler chickens. However, it causes laying birds to retain egg yolk and omphalitis in hatched chicks **(Munang'andu et al. 2012)**

A potential source of human infection by a pathogenic strain of *E. coli* is food animals, particularly poultry, as well as poultry houses **(Stromberg et al. 2017).** Some of those lethal illnesses include meningitis, endocarditis, urinary tract infection, and septicemia **(Daini et al. 2005).** One of the main reasons why it is difficult to effectively treat diseases with antimicrobial drugs is because of bacterial antimicrobial resistance. Resistance of bacterial strains is provoked by modifications those done by bacteria in order to resist antibacterial agents through inactivation of them or by decreasing their therapeutic efficacy. Through time, the resistance appears spontaneously in microorganisms due to mutations of genes. The abuse and misuse of antibiotics in treatment are enhancing the existence of such mutations **(Aslam et al. 2018).** The spread of antimicrobial-resistant *E. coli* from chickens to humans can occur directly or via food. The resistant bacteria may become colonized in the human gastrointestinal tract which may also contribute resistance genes to the human endogenous microflora **(Amir et al. 2019).**

Nanoparticles that are metal-based declared to be the most popular inorganic nanoparticles and represent a favorable solution against the antibiotic resistance. They use completely unique mechanisms of action in compared to those mediated by antibiotics and they have the ability to exhibit anti-bacterial action against which already have resistance. Moreover, the targeting of multiple biomolecules which is a unique feature they are possessing is compromising the formation of resistant strains **(Slavin et al. 2017).** Nanotechnology science is dealing with the synthesis and stabilization of substances at nanoscale, such substances have their size ranging from 1 to 100 nm **(Moore, 2006).** Nanomaterials have distinct and size-related physicochemical characteristics which are significantly aiding in how effective are their implementations in different applications **(Fabrega et al. 2011).** Sliver nanoparticles are announced to be one of the most commonly utilized nanoparticles **(Sharma et al. 2009).** Nowadays, silver nanoparticles have been implemented in a wide range of applications at various fields such as biolabeling, sensors and antimicrobial agents due to their unique physiochemical and biological features **(Rai et al. 2009; Ingle et al. 2008; Kim et al. 2008; Pal et al. 2007; Bhainsa and Souza, 2006).** The medical properties of silver have been popular since a long time ago. Silver-based substances have been utilized in many antimicrobial applications. It is an established fact that silver ions are very toxic to microorganisms which involve over a 10 major species of bacteria. There is a huge increase in the surface area available for the microorganism to be exposed to because of size-related properties **(Slawson et al. 1992; Zhao et al. 1998).**

The goal of conduction our research is to assess the effectiveness of silver nanoparticles those synthesized by utilization of *Aspergillus niger* as an anti-bacterial agent against *E.coli* bacteria that have been isolated from chickens.

2. Materials and methods

2.1. Sampling

2.1.1. Sampling sites

A total number of 75 samples have been gathered in the form of cloacal swabs, intestinal fluid and faecal material from the chickens at different ages. The dead birds from the field were presented for P.M. examination at AHRI and the suspected samples were collected.

2.1.2. Cloacal swab sampling

The samples were collected using a sterile swab stick that has been gotten moistened by 0.9% sodium chloride solution. The swab stick was positioned into the cloacae of the chicken and putted in sterile vials.

2.1.3. Intestinal fluid *sampling*

After P.M examination of suspected cases, intestines have been collected and each intestine was positioned separately from other ones into a sterile jar that has 500 ml of 0.9% sodium chloride solution in it. The suspended fluid

of 0.9% sodium chloride solution has been further used for the processes of isolation and identification.

2.1.4. Faecal material sampling

Five grams samples were collected from poultry houses and putted into sterile vials with the aid of sterile cotton buds. The samples were relocated into crew-caped test tubes which have 10 ml of nutrient broth in them.

2.1.5. Sample transfer

All of the samples have been transferred right away to the laboratory in a foam box with ice.

2.2. Isolation and identification of *E.coli*

A subculturing has been done to the selective enrichment broth which is previously incubated by using the samples of cloacal swab and faecal material. The subculturing involves the transfer of a loop full from selective enrichment broth to Eosine Methylene Blue (EMB) agar medium. In addition to that, 1 ml sample of intestinal fluid has been settled into sterile plates which were then mixed with sterile Eosine Methylene Blue medium (EMB) and poured into plates after leaving them to cool to about 45^{o}C. Identification was done through gram staining technique and several biochemical tests which include oxidase, catalase, citrate, indole, Voges-Proskauer test, methyl red test, urease, nitrate reduction, and sugar fermentation **(Muhammad et al. 2009).**

2.3. Biosynthesis of silver nanoparticles from *Aspergillus niger*

2.3.1. Formation of the biomass

In order to form the biomass, fungi have been exposed to aerobic conditions to make them grow. The liquid media in which fungi were allowed to grow in is contained in gram per liter Potassium dihydrogen phosphate, 7; Potassium hydrogen phosphate, 2; magnesium sulfate heptahydrate, 0.1; Diazanium sulfate, 1; yeast extract, 0.6; and glucose, 10. The flasks have been settled on the orbital shaker at a speed of 150 rpm at 25◦C.

2.3.2. Silver nanoparticles biosynthesis

Twenty g of the prepared biomass has been mixed with 200 ml of Milli-Q deionized for 48 hours at 25°C in a flask and settled on orbital shaker at a speed of 150 rpm. The cell filtrate was prepared by passing the content through Whatman filter paper no. 1. For the sake of silver nanoparticles production, an exact quantity of Silver nitrate has been added to the cell filtrate in a flask to produce silver ion (Ag+) in a concentration of 1 millimolar then agitated at 25◦C without exposing to light **(Kamiar et al. 2016).**

2.4. Characterization techniques of silver nanoparticles

A lot of characterization techniques have been executed to give the physicochemical report of silver nanoparticles. The techniques involved the use of Fourier-transformed infrared (FT-IR) which is used in identification of the chemical bonds through the formation of the infrared absorption spectrum, Transmission electron microscopy (TEM), and scanning electron microscopy (SEM) to reveal the morphology and visualization of the nanoparticles.

2.5. Assessment of the anti-bacterial activity of silver nanoparticles

The inhibition zone assay has been done through utilization of the agar well diffusion method. Colonies that have been grown overnight on an agar plate were used in the sake of preparation of the inoculum suspension that adjusted at 0.5 McFarland then the suspension was used to inoculate on Mueller-Hinton agar to carry out the inhibition zone assay. The tested compound was having a concentration of 50 mg/ml using dimethyl sulfoxide (DMSO) as a solvent, dimethyl sulfoxide (DMSO) was used as a control in this assay. The inhibition zone was measured around each well after 24h at 37^{o}C **(Hindler et al. 1994).**

3. RESULTS

3.1. Isolation and identification of *E.coli*

3.1.1. Number of *E.coli* strains isolated and identified

Table 1. Number and type of samples in which *E.coli* strains have been isolated and identified.

Sample type	No. of samples	No. of *E.coli* strains isolated and identified	% of *E.coli* strains isolated and identified
Cloacal swabs	25	17	68%
Intestinal fluid	25	22	88%
Faecal material	25	14	56%
Total	75	53	70.6%

3.1.2. Biochemical identification of E.coli

Table 2. Biochemical examinations which were carried out to identify *E.coli.*

Biochemical tests	Results
Urease test	-ve
Methyl red test	+ve
Catalase	+ve
Citrate utilization test	-ve
Indole test	+ve
Triple sugar iron test (H_2S production)	+ve
Voges- Proskuer test (VP)	-ve
Oxidase test	-ve
Sugar fermentation test	+ve
Nitrate reduction test	+ve

3.2. Characterization of silver nanoparticles

3.2.1. Fourier transformed infrared (FT-IR)

Infrared absorption spectrum has demonstrated the peak range in the 500-4000 cm-[1]. The Results revealed sharp absorption peaks at 548.59, 1638, 2074 and 3452 cm-1 which are related to several functional groups.

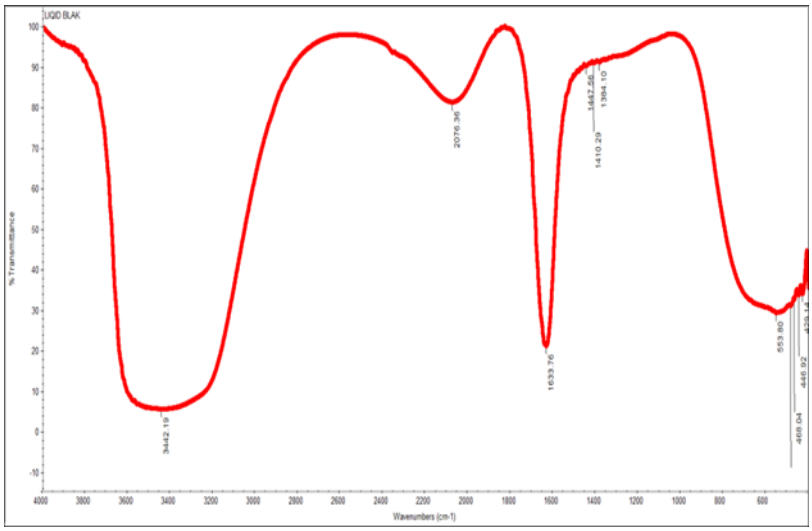

Figure (1) displays the FT-IR spectrum of biosynthesized silver nanoparticles

3.2.2. Transmission electron microscopy (TEM)

Transmission electron microscopy was one of techniques that used to reveal the size and shape of the silver nanoparticles. The results showed that silver nanoparticle have a spherical shape with average size range of 12–16 nm.

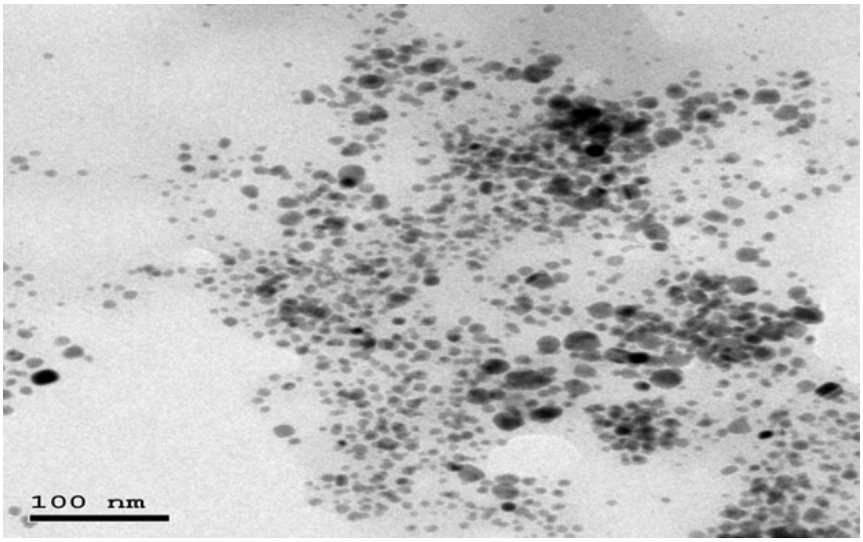

Figure 2. shows TEM image of silver nanoparticles which revealed the average size of silver nanoparticles.

3.2.3. Scanning electron microscopy (SEM)

In addition to TEM, scanning electron microscope was further done to confirm the morphology of the silver nanoparticles. The results confirmed the spherical shape of silver nanoparticles

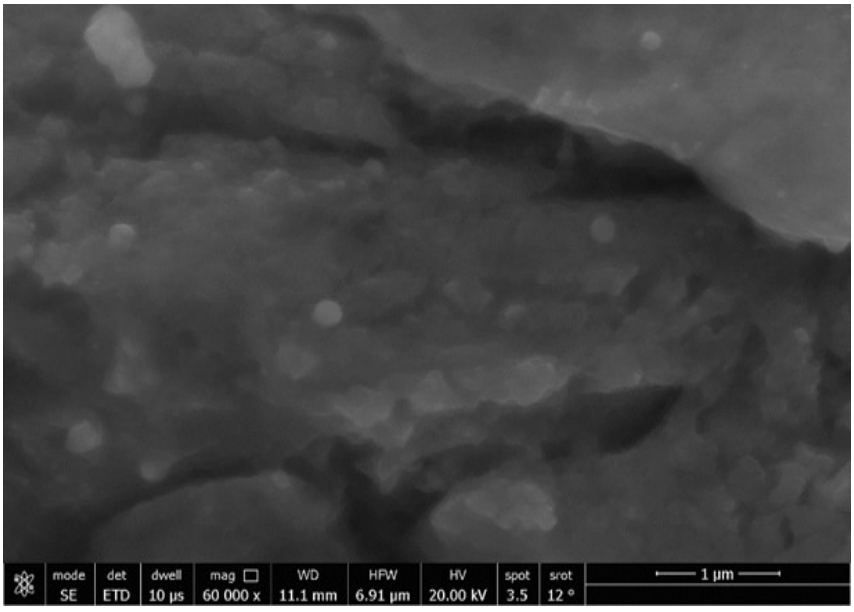

Figure 3. demonstrates the SEM image of silver nanoparticles

3.3. Assessment of the anti-bacterial activity of silver nanoparticles

In order to obtain MIC value and inhibition zone diameter, the agar well diffusion test has been executed and the results were obtained (table 3) (figure 1).

Table 3: The results of Inhibition zone and MIC obtained through agar well diffusion method.

Tested microorganism	Inhibition zone diameter (mm)	MIC value (µg/ml)
E.coli	14	39.06

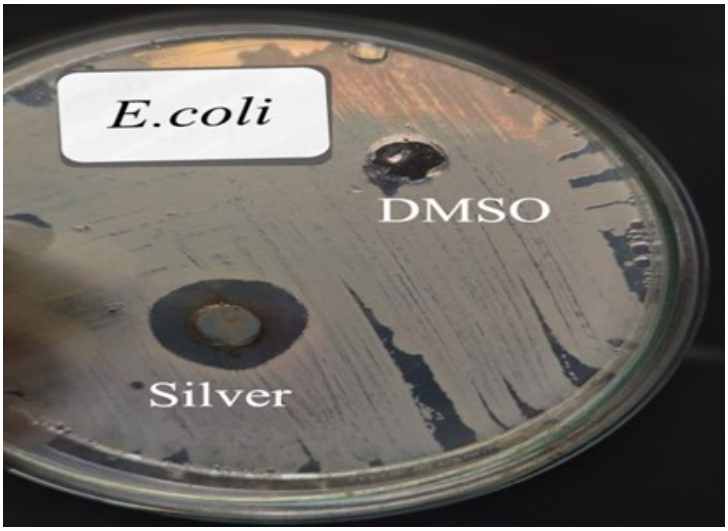

Figure 4. Displays the inhibition zone diameter that resulted from the anti-bacterial action of silver nanoparticles against *E.coli*. Dimethyl sulfoxide has been used as a control.

DISCUSSION

E. coli is commonly found in human and animal intestinal tracts. The faecal contamination or contamination during animal slaughtering can result in a high probability of its existence in the soil, water, and foods. *E. coli* can be considered as pathogenic bacteria; for instance, certain strains of *E.coli* can engage in the pathogenesis process of causing colibacillosis in poultry, on the other hand, they can also cause severe human diseases, like colitis and hemolytic uremic syndrome **(Chansiripornchai 2009; Ferens and Hovde 2011).** In this study, the incidence of *E.coli* in different samples of chicken is 70.6%. This result is similar to the results described by **(Jakaria et al. 2012).**

While a higher percentage (91.8%) was detected by **(Derakhshanfar and Ghanbarpour 2002).** Nowadays, a lot of researchers have reported the danger of antimicrobial resistance existence which is raising global concerns as it involves the unresponsiveness of bacteria to the lethal doses of antibiotics which in a result make a requirement for the invention of new methods to treat diseases which caused by bacteria **(Desselberger, 2000; Muhammad et al. 2009).** The use of nanotechnology science in order to produce the nanoparticle form of substances which have a high potential to be bactericidal has shown a huge enhancement of the antimicrobial effect of those substances. A high number of studies have announced how important is the idea of using non-traditional antimicrobial agents which are nanotechnological-based to solve the problem of antimicrobial resistance **(Rai et al. 2012).** In the sake of conducting our study, evaluation of the effectiveness of silver nanoparticle's anti-bacterial action on *E.coli* bacteria isolated from different samples of chickens. In regard of the Biosynthesis of silver nanoparticles, a green chemistry method has been used to afford a clean, safe, and cost-effective way for the production of nanoparticles. The method involved a biosynthesis of nanoparticles through utilizing of *Aspergillus niger* to aid in formation of silver nanoparticles and the process of the biosynthesis agreed with what has been stated before in the

other research **(Kamiar et al. 2016).** Concerning with the characterization of silver nanoparticles, the physicochemical report of the biosynthesized silver nanoparticles has been obtained through utilizing different techniques, such as Fourier transformed infrared(FT-IR), Transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The execution steps of those techniques were quietly similar to the acquired information from preceding papers **(Nuha, 2023).** The anti-bacterial activity which is possessed by silver nanoparticles has been assessed through agar well diffusion method **(Seetharam et al. 2018)** using dimethyl sulfoxide (DMSO) as a control then the values of both MIC and inhibition zone diameter have been determined.

The silver nanoparticles could inhibit the isolated *E.coli* strains compared with the control. This result is agreed with the results described previously by (**Bansal, et al. 2010; Gaikwad Sagar and Bhosale Ashok 2012).**

CONCLUSION

In our research work, the isolation of *E.coli* from chicken through sampling from cloacal region, intestinal fluid, and faecal material was carried out and then identified through biochemical examinations(catalase, urease, oxidase, indole and methyl red test). Silver nanoparticles have been synthesized using a green chemistry approach through utilizing *A spergillus niger* in the production process of nanoparticles. The physicochemical characteristics have been acquired through Fourier transformed infrared (FT-IR), Transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The anti-bacterial effect of silver nanoparticles has been evaluated through agar well diffusion test and the values of MIC and inhibition zone were noted.

Data Availability Statement

The original contributions presented in the study are included in the article Material; further inquiries can be directed to the corresponding author.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgments

The authors would like to thank The Animal Health Research Institute for the support provided for this work.

Author Contributions

Conceptualization: Flourage M.Rady, Gamal A.M Omran; Formal analysis: Eman.F.E; Funding acquisition: Flourage M.Rady, Gamal A.M Omran, Eman.F.E; Investigation: Gamal A.M Omran; Methodology: Eman.F.E; Project administration: Flourage M.Rady; Resource: Eman.F.E, Gamal A.M Omran; Supervision: Flourage M.Rady; Validation: Gamal A.M Omran; Visualization: Eman.F.E; Writing original draft: Flourage M.Rady; Writing -review edition: All authors have confirmed the final version of the manuscript.

REFERENCE

Amir M, Riaz M, Chang YF, Akhtar ., Nadeem H, Ahmad Z, Nadeem M. 2019. Spread of antibiotic resistant Escherichia coli from broiler to human populations. Pakistan Journal of Agricultural Sciences., (56): 977–983.

Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU. 2018. Antibiotic resistance: A rundown of a global crisis. Infect. Drug Resist. (11): 1645–1658.

Bansal V, Li, V, O'Mullane AP, Bhargava SK. 2010. Shape dependent electrocatalytic behaviour of silver nanoparticles. Cryst. Eng. Comm. (12): 4280–4286.

Bhainsa KC, SF, D'Souza 2006. Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus. Colloids and Surfaces B: Biointerfaces, 47 (2): 160–164.

Chansiripornchai N. 2009. Comparative efficacy of enrofloxacin and oxytetracycline by different administration methods in

broilers after experimental infection with avian pathogenic Escherichia coli. Thai Journal of Veterinary Medicine (39): 231 –236.

Daini OA, Ogbulo OD, Ogunledun A. 2005. Quinolones Resistance and R-plasmids of some gram negative enteric Bacilli. Afr. J. Clin. and Exp. Micro. (6): 14-19.

Derakhshanfar A. Ghanbarpour R. 2002. A study on avian cellulitis in broiler chickens. Veterinarski arhiv, 72(5): 277-284.

Desselberger U. 2000. Emerging and re-emerging infectious diseases. J.Infect. 40,3 15. doi: 10. 1053 / jinf .1999.0624.

Fabrega J, SN, Luoma CR, Tyler TS, Galloway JR, Lead 2011. Silver nanoparticles: behaviour and effects in the aquatic environment. Environment International, 37 (2): 517–531.

Ferens WA, Hovde CJ. 2011. Escherichia coli O157:H7: animal reservoir and sources of human infection. Foodborne Pathogens and Disease (8):465–485.

Gaikwad Sagar and Bhosale Ashok 2012. Green Synthesis of Silver Nanoparticles Using Aspergillus niger and Its Efficacy Against Human Pathogens. European Journal of Experimental Biology, 2 (5):1654-1658.

Hindler JA, Howard BJ, Keiser, JF. 1994. Antimicrobial agents and Susceptibility testing. In: Howard BJ(Editor), Clinical and pathogenic Microbiology. Mosby-Year Book Inc., St. Louis, MO,USA.

Ingle A, A, Gade S, Pierrat C, S¨onnichsen M, Rai. 2008. Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Current Nanoscience, 4, (2): 141– 144.

Jakaria A, Ariful MI, Minara MKh. 2012. Prevalence, Characteristics and Antibiogram Profiles of Escherichia coli Isolated from Apparently Healthy Chickens in Mymensingh, Bangladesh. Microbes and Health, 1 (1): 27-29.

Kim KJ, WS, Sung SK, Moon JS, Choi JG, Kim DG, Lee. 2008. Antifungal effect of silver nanoparticles on dermatophytes. Journal of Microbiology and Biotechnology, 18(8):1482–1484.

Kamiar Zomorodian, Seyedmohammad Pourshahid, Arman Sadatsharifi, Pouyan Mehryar, Keyvan Pakshir, Mohammad Javad Rahimi & Ali Arabi Monfared, 2016. Biosynthesis and Characterization of Silver Nanoparticles by Aspergillus Species. Hindawi Publishing Corporation. BioMed Research International. Volume 2016, Article ID 5435397, 6 pages.

Moore MN. 2006. Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? Environment International, 32(8): 967–976.

Muhammad Ali Akond Saidul Alam SMR, Hassan Momena Shirin. 2009. Antibiotic Resistance of Escherichia Coli Isolated From Poultry and Poultry Environment of Bangladesh. Internet Journal of Food Safety, (11): 19-23.

Munang'andu HM, Kabilika SH, Chibomba O, Munyeme M, Muuka GM. 2012. Bacteria Isolations from Broiler and Layer Chicks in Zambia. Journal of Pathogens, 1-6.

Nuha M, Alhazmi. 2023. Fungicidal Activity of Silver and Silica Nanoparticles against Aspergillus sydowii Isolated from the Soil in Western Saudi Arabia. Microorganisms, 11, 86. https://doi.org/10.3390/microorganisms11010086

Pal SYK, Tak JM, Song. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli, Applied and Environmental Microbiology, vol. 73(6):1712–1720.

Rai MA, Yadav A, Gade. 2009. Silver nanoparticles as a new generation of antimicrobials," Biotechnology Advances, vol. 27, (1): 76–83.

Rai MK, Deshmukh SD, Ingle AP, Gade AK. 2012. Silver nanoparticles: the powerful

nano weapon against multidrug-resistant bacteria. J. Appl. Microbiol. 112,841 852.doi:10.1111/j.1365-2672.2012.05253.

Seetharam Charannya Dasarathan Duraivel Krishnan Padminee Saravanan Poorni Cruz Nishanthine & Manali Ramakrishnan Srinivasan. 2018. Comparative Evaluation of Antimicrobial Efficacy of Silver Nanoparticles and 2% Chlorhexidine Gluconate When Used Alone and in Combination Assessed Using Agar Diffusion Method: An In vitro Study. Contemporary Clinical Dentistry | Volume 9 | Supplement 2 | September 2018 (PMID: 30294145).

Sharma VK, RA, Yngard Y, Lin. 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145(1-2): 83–96.

Slavin YN, Asnis J, Häfeli UO, Bach H. 2017. Metal nanoparticles: Understanding the mechanisms behindantibacterial activity. J. Nanobiotechnol. (15):1–20.

Slawson RM, Trevors, JT Lee, H. 1992. Silver accumulation and resistance in Pseudomonas stutzeri. Arch. Microbiol. (158): 398–404.

Stromberg ZR, Johnson JR, Fairbrother JM, Kilbourne J, Van Goor A, Curtiss R. 3rd Mellata M. 2017. Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. PloS One, 12, e0180599.

Zhao GJ, Stevens SE. 1998. Multiple parameters for the comprehensive evaluation of the susceptibility of Escherichia coli to the silver ion. Biometals (11): 27–32.