Readers Insight
Publisher of open access books and journals

PJMLS
Online ISSN: 2707-4471. Print ISSN: 2707-448X

Review Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v4iSp I.2131	Copyright © All rights are reserved by Corresponding Author
Vol. 4 No. Sp.1, 2021: pp. S96-S104	
www.readersinsight.net/pjmls	
Submission: November 23, 2021	**Revised:** December 19, 2021 **Published Online:** December 27, 2021

BIOCIDAL ACTIVITY OF SILVER NANOPARTICLES AGAINST *ESCHERICHIA COLI*

Sana Saeed Ahmad[1*], Umbreen Shaheen[1], Abdul Samad[2], Sidra Aftab[1], Farha Manzoor[1], Farkhanda[1], Irfan Shahzad Sheikh[2]

[1]**Department of Zoology,** University of Balochistan, Quetta, Pakistan

[2]**Center for Advanced Studies in Vaccinology & Biotechnology (CASVAB),** University of Balochistan, Quetta, Pakistan

*Corresponding Author: Sana Saeed Ahmad. E. mail: sanasahmad1994@gmail.com

Abstract

Nanotechnology could be particularly useful in the treatment of bacterial illnesses. Silver nanoparticles (AgNPs) are progressively being employed to target bacteria, as a substitute to antibiotics. Escherichia coli (E. coli) is a gram-negative bacteria present in the environment, foods, and intestines of animals and humans. Some of its strains have the potential to cause illness in humans, some roots diarrhea, while others cause pneumonia, lungs illness, urinary tract infections, and a number of other problems. The antibacterial activity of AgNPs against E. coli has been widely documented in the literature. The biocidal activity of Silver nanoparticles is dependent on their size, stability, and concentration given to the growth medium, as this allows for a longer period of interaction between bacteria and nanoparticles. As a result, the particle size has a significant impact on the functional activities of nanoparticles. Although the mechanism of action of AgNPs on bacterial membranes is yet unknown, morphological and structural alterations in bacterial cells have been postulated as a probable mechanism of action. As a result, the attachment of AgNPs to bacterial surfaces is crucial in the dispute over NPs bacterial cytotoxicity. Structural changes in bacterial cell membrane have been observed by many researchers. Bacterial cells leads up to damage and eventual cell death after AgNps treatment. Novel biocidal agents have been developed as a result of recent breakthroughs in nanotechnology, specifically the ability to manufacture highly ordered AgNPs of any size and shape. The aim of this review paper was to highlight the microbial activity of silver nanoparticles on E. coli.

Keywords: *Nanotechnology, Silver nanoparticles (AgNPs), Escherichia coli (E. coli), Antibacterial, Minimum inhibitory concentration (MIC), Zone of inhibition (ZOI)*

INTRODUCTION

NANOTECHNOLOGY AND NANOPARTICLES (NPS)

Richard P. Feynman in 1959 presented Nanotechnology in his illustrious speaking ''There's Plenty of Room at the Bottom'' (1). This ground has seen a variety of inventive developments (2). Nanotechnology is the development, composition, and management of NPs (elements) with sizes ranging from 0.1 to 100 nm. Because of their nanoscale dimensions and great surface to volume relationship, NPs have exclusive physical and chemical properties (3). The ocular and physicochemical characteristics of these particles can be changed by simply modifying their size. For example, the luminescence emulsion properties of Cadmium selenide (CdSe) NPs may be altered by changing their size; a particle with a size of 2 nm emits blue light, whereas a particle with a size of 6 nm emits red light (4). These materials can be 0D, 1D, 2D, or 3D depending on the overall shape (5).

CLASSIFICATION OF NPS

NPs can be classified into numerous classes based on their compositions, physical shapes, and dimensions (5).

Metal NPs (made of metal precursors such as copper, silver, and gold), polymeric NPs (organic based), ceramic NPs (inorganic nonmetallic solids), and fullerenes (nanomaterials which are composed of spherical resonating enclosure for example, allotropes of carbon) are the different types of NPs (6).

APPLICATIONS OF NANOTECHNOLOGY

The inimitable properties of NPs and other nanomaterials have been used by medical and biological research societies for a variety of applications over the last few decades. For example, NPs are used for contrasting effects in animal cell imaging and in the medical field for cancer treatment (7).

New promises and opportunities for nanotechnology exploration have emerged as a result of many unique expressions. Biology and nanotechnology have a natural relationship. Nanotechnology has enabled the expansion of overprovision of particles with various belongings (electrifying, magnetic, and optical). By combining NPs or nano elements with different structures and bio molecules, we can add different capabilities to them. For example, magnetic NPs combined with the antibody Herceptin are used in cancer treatment. These NPs are employed as a contrast agent for tumour cell imaging (Herceptin directs the magnetic NP towards the tumour cells, while the magnetic NPs make a gesture to represent detection) (8). The combination of biology and nanomaterials is guiding advances in the development of a variety of different agents, diagnostic equipment, diagnostic tools, therapies, and drug delivery vehicles (9). Hirsch *et al*., 2003 have pioneered the use of metallic nanostructures in cancer treatment (10). There have been numerous advancements in the usage of nanomaterials in cosmetics. Cosmetics with nanomaterials have better stability and sensory qualities (11). Nanotechnology has novel uses in domains such as molecular biology, genetics, and forensic science. Nanotechnology is employed in the prevention, analysis, and treatment of several ailments, furthermore, in the treatment of water and drug delivery (12). Nanotechnology offers a viable alternative for developing new antibacterial materials or bactericides (13).

SILVER NANOPARTICLES (AGNPS)

AgNPs are one of the most widely utilized NPs, they are used as antibacterial agents for medical applications (14). Nanosilver is a novel class of material having different physiochemical and biological properties than bulk materials, these properties include improved optical, electromagnetic, catalytic, and antibacterial activity (15). Cryochemical production, solution radioactivity, electrochemical reduction and spark discharging have all been used to turn metallic silver into ultrafine particles, resulting in nanosilver. Nanosilver particles are typically less than 100 nm in size and contain between 20 to 15000 silver atoms. AgNPs come in a variety of forms, including cubes, spheres and rods. Nanostructures can also be created as wires, films and tubes (16).

USES OF AGNPS

Because of their exceptional optical, microbiological, electrical, and chemical capabilities, the application of AgNPs is quickly growing in the twenty-first century. Drug delivery, pathology, bioscience, pathogen detection, catalysis, tumour detection, diagnostics, wound healing, and antimicrobials are just a few of the applications for AgNPs (17, 18). Aspect ratio, crystal size, crystalline density, and shape all influence the characteristics of NPs (19, 20). Because of their greater aspect ratio, uniformly distributed and narrow sized NPs have better chemical and physical characteristics (21). Regardless of the synthesis procedure, AgNPs have a very high aspect ratio, which influences surface attributes such as solubility and stability. AgNPs with a high aspect ratio are required for a variety of applications, including catalysis, microbial resistance, and so on (22). The use of AgNPs in medicine can be classified into two categories: diagnostic and therapeutic. Lim *et al*, discovered that AgNP-based Surface Enhanced Raman Spectroscopy (SERS) can be utilized to diagnose cancer in a non-invasive manner. In the not-too-distant future, this method of cancer diagnosis will become an unavoidable aspect of cancer detection. AgNPs are now commonly used in medical research. Wound dressing, scaffolds, eye treatment, and dental hygiene (23), as well as bone substitute biomaterials (24).

Textile companies are now using nanomaterials commercially (25). NPs are either woven into the material or coated on it. T-shirts, athletic clothing, undergarments, and socks are among those things that contain AgNPs (26).

Optical purposes are likewise served by AgNPs. Solar cells, medical imaging, optical limiters, plasmonic devices, and more applications employ it. AgNPs are also used in LCDs, high-intensity LEDs, and touch screens (27).

AgNPs are commonly employed in the food business due to their antimicrobial properties and lack of preservatives. Human cells are unaffected by low concentrations of AgNPs, while the majority of viruses and bacteria are killed. As a result, it is widely utilized in the purification of food and water in daily life, as well as an infection resistive in medicine. Food packaging contains NPs. One of the commercially available bags with AgNPs is the Sun River industrial Nano silver fresh food bag (27). Because of their antifungicidal and antibacterial properties, AgNPs are frequently utilized in consumer products such as soaps, food, plastics, pastes, and textiles (28). The biocidal effect of several nanomaterials such as titanium alginate, copper, zinc, and AgNPs has been studied, with AgNPs demonstrating the strongest antibacterial activity against viruses, bacteria, and a variety of other microorganisms (29). AgNPs are employed to kill germs because of their bactericidal properties. These particles penetrate bacteria's cell membrane and form a tight bond with the thiol groups of active enzymes, deactivating them (30, 31). Experimental research suggests that when bacteria are exposed to AgNPs, they lose their ability to replicate. AgNPs cause changes in the structure of the bacterial cell membrane, resulting in the formation of small electron-dense pieces (32, 33). According to nanotechnologists, nanosilver products are particularly effective against microorganisms due to the effects of nanosilver elements such as: Nanosilver kills 99 percent of bacteria and roughly 650 types of dangerous/harmful germs in 30 minutes, which is 2 to 5 times faster than conventional forms of silver (15). Because of the large fraction of Ag-atoms present on the surface of AgNPs and the highly precised surface areas, AgNPs have a strong antibacterial activity, whereas silver in metallic form has a lower antibacterial activity (29). The antimicrobial potency of AgNPs may be influenced by the characteristics and individuality of some bacterial classes. Gram-negative and gram-positive bacteria devour various structural and physical alterations in their cell membranes; the key difference is the viscosity of the murein coating, and this is the cause for AgNPs limited efficiency against *Staphylococcus aureus*. However, because of the electrostatic interaction between NPs (which have a positive charge) and bacteria's cell membrane, the positive (+ive) charge on silver ion is critical for its antibacterial activity (which has negative (-ive) charge on it) (29). The biocidal action of AgNPs against bacteria is reliant on their concentration. AgNPs penetrate the bacterial membrane, causing the membrane to become permeable and the cell to die. Metallic depletion in the outer membrane causes random creation of shaped pits, and Ag-NPs alter the membrane's permeability (34). As AgNPs collect in the bacterial cell, a less molecular weight region forms in the middle, and the bacteria attempts to protect its DNA from the Ag ions. When silver ions are liberated from Ag-NPs, they boost antibacterial activity. The bactericidal efficiency of NPs is determined on the particle's shape. NPs in a truncated triangle form inhibit bacteria with a silver content of 1 g, whereas spherical NPs require the complete silver amount of 12.5 g. When particles are rod-shaped, a silver content of 50 to 100 ug is necessary. As a result, different types of Ag-NPs have diverse impacts on bacteria (29). The bactericidal activity of NPs on bacteria may be attributed to the breakdown of the organism's enzymes or plasma membrane. Cell death is caused by a reduction in metabolic pathways and the outflow of cytoplasm content into the surrounding environment (16). According to Zhang XF *et al.* (2016), the reduced size of AgNPs can root additional pathogenicity to bacteria and have a stronger antimicrobial result than bigger particles since they have a higher surface area (35).

ESCHERICHIA COLI (E. COLI)

E. coli is a gram negative bacterium which is present in the environment, foods, and the intestines of both animals and humans. Different strains of *E. coli* exist. Some of its strains have the potential to make us sick. Some varieties induce diarrhea, while others can cause pneumonia, respiratory sickness, urinary tract infections, and a variety of other disorders (16). *E. coli* is a naturally occurring bacterium found in the birds,

guts of people, and other animals which are warm blooded, and is commonly used as a marker for faecal pollution of water. It's a tough microbe that can adapt to environmental challenges genetically and has been shown to survive and multiply in the wild (36, 37).

Discussions have been upraised in current years about *E. coli*'s classification as a faecal indicator microbe, based on the foregoing (38). Despite the fact that most *E. coli* strains are interdependent, harmful *E. coli* strains can include a number of virulence factors and cause a variety of diseases (39). Infectious *E. coli* can be categorized as extraintestinal pathogenic *E. coli* (ExPEC) or intestinal pathogenic *E. coli* (InPEC) based on the presence of certain virulence factors. ExPEC strains are related to infection of the urinary tract, septicaemia and meningitis in newly born babbies, because they can root problems in the areas which are outside of the intestine. ExPEC can make colonies in the intestinal tract without causing any problem, just as commensal *E. coli*. On the other hand InPEC strains can root diverse kinds of infectious diarrhea and can be distributed into 6 groups: enterohaemorrhagic (EHEC); enteropathogenic (EPEC); enteroaggregative (EAEC); enterotoxigenic (ETEC); enteroinvasive (EIEC) and diffusely adherent *E. coli* (DAEC). Every form of InPEC possesses its own set of infection processes and symptoms (40).

The number and severity of foodborne ailment epidemics connected to pathogenic *E. coli*, is on the rise (41). Consequently, *E. coli* is recognized as a developing pathogen. Contaminated irrigation water is the most prevalent way because of which this microbe can be introduced to vegetables. The World Health Organization (WHO) and the South African Department of Water Affairs (DWA) have fixed a suggested edge of 1000 faecal coliforms/100 mL for water which is used for irrigation of the fresh crops (vegetables), recognising the possible threat (42, 43). When *E. coli* passes in the water system, its spread is influenced by a number of geohydrological parameters, including the volume of particles present and the rate of flow (44). Current research has revealed that when *E. coli* is present in the atmosphere, it is proficient of multiplying in a variety of situations (45). *E. coli* appears to be capable to develop in limited places and persist when the atmosphere is augmented with faecal material (46, 47). Since they turn as numerous reservoirs where irrigation or rain water can serve to reinstate faecal pollutants into the nearby surrounding, the occurrence and perseverance of *E. coli* in the atmosphere increase weighty distresses in pursuing the distribution design through ailment epidemics. Because of this, *E. coli* trait is also concerning the feast of antibiotic confrontation. Antibiotic conflict to minimum one disinfectant was found in 24 percent of the 600 isolates tested in a research of water sources related with cattle farming, and many were resistant to multiple antimicrobial agents (48). Because of the extensive incidences of illness eruptions linked to water sources polluted with harmful forms of *E. coli*, the occurrence of *E. coli* in the surrounding offers both instant and long-term distresses about the organism's persistence as it narrates to the spread of antibiotic confrontation (49).

EFFECT OF AGNPS ON *E. COLI*

The biocidal activity of AgNPs against Gram-negative bacteria is reliant on their concentration. AgNPs penetrate the bacterial membrane, causing the membrane to become permeable and the cell to die. Metallic depletion in the outer membrane causes random creation of shaped pits, and Ag-NPs alter the membrane's permeability. When *E. coli* is exposed to AgNPs, its membrane structure deteriorates (34).

Morones *et al.* (2005), identified that AgNPs work against Gram-negative bacteria *(E. coli)* in three ways: (i) NPs in the size array of 1–10 nm confer to the surface of cell membrane and significantly disrupt its appropriate function, such as penetrability; (ii) They have the ability to breach inside bacteria and inflict additional destruction by reacting with sulphur and phosphorus-containing molecules like DNA; (iii) NPs produce silver ions, that contribute to the bactericidal effect of AgNPs like those described by Feng QL (32, 50).

The use of AgNPs as an antibacterial agent was confirmed against selected Gram-negative bacteria including *E. coli* on liquid media and agar plate. The results disclosed that the tested bacteria might utterly obstruct by AgNPs in a squatter time at low-slung concentration. This could be because of Gram-negative bacteria's cell wall construction. Gram-negative bacteria have a unique cell wall construction that differs

from Gram-positive bacteria. Gram-negative bacteria have a cytoplasmic membrane, a thin peptidoglycan layer, and a lipopolysaccharide-based outer membrane (51).

Many researchers have investigated the antibacterial properties of AgNPs in contradiction of multidrug resistant bacteria, and it has been confirmed that AgNPs are useful against multidrug resistant bacteria such as multidrug resistant *E. coli* (52, 53).

Ansari MA *et al.* (2013), used SEM to examine the ultra structural changes in the cells of *E. coli* (before and after their interaction with AgNPs). Damage to the cell surface was not identified in natural *E. coli*, which was normally of rod shape with a plane superficial layer. In the AgNPs-treated group, however, irregular fragments emerged instead of normal rod-shaped cells. The treated *E. coli* cell was rigorously smashed, with multiple depressions and indentation, and the original rod like shape had inflated to a larger size; considerable damage of membrane reliability was also examined, indicating cell membrane and cell wall damage (54). When observed by High resolution transmission electron microscopy (HR-TEM), Untreated *E. coli* cells looked to have an ordinary internal structure, with a multifaceted cell surface comprised of an external membrane, a peptidoglycan layer in the periplasmic region, and a cytoplasmic membrane. Temporarily, the cells of bacteria looked to be severely harmed after being exposed to AgNPs. The cells had an abnormal shape, cracking and rupturing. Around injured bacterial cells, electron-dense particles called precipitates were also discovered. The detachment of the cell membrane from the cell wall in smashed cells was localized or widespread. In the injured cells, cellular deterioration was supplemented by electron-translucent cytoplasm and cellular disintegration. The NPs anchor the cell in multiple locations and cause damage to the membrane at various locations, perhaps leading to cell death (54).

In order to assess AgNPs antibacterial action against Gram-negative bacteria, the growth kinetics of *E. coli* were examined by Eloise I. Prieto and Analiza A Kiat, (2017). AgNPs had antibacterial action that was dosage dependent and inhibited the development of cultured cells (55).

Many scientists have studied AgNPs and their antibacterial efficacy against *E. coli*. The minimum inhibitory concentration (MIC) was also analyzed. Zone of inhibition can be also measured by inoculating *E. coli* on Muller Hinton agar (MHA) and applying AgNPs by disk diffusion/ Kibry–Bauer method and well diffusion method. MIC can also be examined by dilution method (Table I and II).

Table I. Effect of AgNPs on *E. coli*

S.No	TEM size of AgNPs (nm)	Doses of AgNPs (μg/ml)	*Escherichia coli* Strain	Effect	Reference
1	100	60	*Escherichia coli* ATCC/15224	Inhibit	56
2	20-30	3.3 nM	*Escherichia coli* ATCC/43890	Inhibit	57
3	10	1	*Escherichia coli* ATCC/43888	Inhibit	58
4	40-72	30	*Escherichia coli* MTCC/443	Inhibit	59
5	7-11	100	*Escherichia coli* ATTC/25922	Inhibit	60
6	18-34	7.95	*Escherichia coli* ATCC/25922	Inhibit	61
7	15-25	14.1	*Escherichia coli* MTCC/1302	Inhibit	62

TEM: transmission electron microscope

CONCLUSION

AgNPs have revelatory antimicrobial activity against the selected Gram-negative bacteria specially *E. coli*. These particles are the most marketable nanomaterial due to their well-established broad range antibacterial capabilities and decreased tendency to produce microbial resistance. As a result, AgNPs could be a promising candidate for developing as an antibacterial agent against multidrug-resistant bacteria. AgNPs could lead to important discoveries in a variety of fields, including medical devices and antibacterial systems.

Table II. Effect of AgNPs on *E. coli* (on MHA)

S.No	Size of AgNps	Concentration or dose of AgNPs	Method by which AgNPS applied	Organism	ZOI diameter (In mm)	MIC of AgNPs	Reference
1	24nm	NR	Well diffusion	*E. coli*	12	NR	63
2	NR	0.2-33nM (20 µL)	Disk diffusion	*Escherichia. coli* ATCC43890	8	>3.3 nM	64
3	NR	1mM (10 µL)	Disk diffusion	*E. coli*	15	7.8 (µg/mL)	51
4	30-80nm	NR	Disk diffusion	*E. coli*	0.9 ± 0.15	NR	65
5	150nm	NR	Disk diffusion	*E. coli*	1.4 ± 0.2	NR	65
6	25-70	NR	Disk diffusion	*E. coli*	1.1 ± 0.35	NR	65
7	15-50	NR	Disk diffusion	*E. coli*	1.5 ± 0.3	NR	65
8	30-200	NR	Disk diffusion	*E. coli*	0.7 ± 0.3	NR	65
9	85.07 ±	10 µL	Disk diffusion	*E. coli*	17.1	NR	66
10	12.86nm	1mM	Disk diffusion	*E. coli*	3.5	NR	67
11	NR	3mM	Disk diffusion	*E. coli*	3.5	NR	67
12	NR 75nm	5mM	Disk diffusion	*E. coli*	3	NR	67

NR: NOT REPORTED

References:

1. Fagerlund G. Determination of specific surface by the BET method. Matériaux et Construction. 1973;6(3):239-45.
2. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN. Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chemical reviews. 2008;108(6):2064-110.
3. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019;12(7):908-31.
4. Horn T, Huber E, Kreft A, Longerich T, Morton T, Myerson D, Prieto VG, Rosenberg A, Treister N, Washington K, Ziemer M. Biology of blood and marrow transplantation. Biol Blood Marrow Transplant. 2015;30(1e15):1e15
5. Todescato F, Fortunati I, Minotto A, Signorini R, Jasieniak JJ, Bozio R. Engineering of semiconductor nanocrystals for light emitting applications. Materials. 2016 Aug;9(8):672.
6. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry. 2019;12(7):908-31.
7. Jun YW, Huh YM, Choi JS, Lee JH, Song HT, Kim S, Yoon S, Kim KS, Shin JS, Suh JS, Cheon J. Nanoscale size effect of magnetic nanocrystals and their utilization for cancer diagnosis via magnetic resonance imaging. Journal of the American Chemical Society. 2005;127(16):5732-3.
8. Singh P, Kim YJ, Singh H, Wang C, Hwang KH, Farh ME, Yang DC. Biosynthesis, characterization, and antimicrobial applications of silver nanoparticles. International journal of nanomedicine. 2015;10:2567.
9. Gao X, Cui Y, Levenson RM, Chung LW, Nie S. In vivo cancer targeting and imaging with semiconductor quantum dots. Nature biotechnology. 2004;22(8):969-76.
10. Hirsch LR, Stafford RJ, Sershen SR, Halas NJ, Hazle JD, West JL. Nanoshell-assisted tumor ablation using near infrared light under magnetic resonance guidance. Proc Natl Acad Sci. 2003;100:113549-54.
11. Raj S, Jose S, Sumod US, Sabitha M. Nanotechnology in cosmetics: Opportunities and challenges. Journal of pharmacy & bioallied sciences. 2012;4(3):186.
12. Curtis J, Greenberg M, Kester J, Phillips S, Krieger G. Nanotechnology and nanotoxicology. Toxicological reviews. 2006;25(4):245-60.

13. Matsumura Y, Yoshikata K, Kunisaki SI, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Applied and environmental microbiology. 2003;69(7):4278-81.
14. Kandile NG, Zaky HT, Mohamed MI, Mohamed HM. Silver nanoparticles effect on antimicrobial and antifungal activity of new heterocycles. Bulletin of the Korean Chemical Society. 2010;31(12):3530-8.
15. Shahrokh S, Emtiazi G. Toxicity and unusual biological behavior of nanosilver on gram positive and negative bacteria assayed by microtiter-plate. Eur. J. Biol. Sci. 2009;1(3):28-31.
16. Wijnhoven SW, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens EH, Roszek B, Bisschops J, Gosens I, Van De Meent D, Dekkers S. Nano-silver–a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology. 2009;3(2):109-38.
17. Guzmán MG, Dille J, Godet S. Synthesis of silver nanoparticles by chemical reduction method and their antibacterial activity. Int J Chem Biomol Eng. 2009;2(3):104-11.
18. Basu S, Jana S, Pande S, Pal T. Interaction of DNA bases with silver nanoparticles: Assembly quantified through SPRS and SERS. Journal of colloid and interface science. 2008;321(2):288-93.
19. Anandan B, Rajendran V. Morphological and size effects of NiO nanoparticles via solvothermal process and their optical properties. Materials Science in Semiconductor Processing. 2011; 14(1):43-7.
20. Laokul P, Amornkitbamrung V, Seraphin S, Maensiri S. Characterization and magnetic properties of nanocrystalline CuFe2O4, NiFe2O4, ZnFe2O4 powders prepared by the Aloe vera extract solution. Current Applied Physics. 2011;11(1):101-8.
21. Mu Y, Liang H, Hu J, Jiang L, Wan L. Controllable Pt nanoparticle deposition on carbon nanotubes as an anode catalyst for direct methanol fuel cells. The Journal of Physical Chemistry B. 2005;109(47):22212-6.
22. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, Paknikar KM. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology. 2002;14(1):95.
23. Lin J, Chen R, Feng S, Pan J, Li Y, Chen G, Cheng M, Huang Z, Yu Y, Zeng H. A novel blood plasma analysis technique combining membrane electrophoresis with silver nanoparticle-based SERS spectroscopy for potential applications in noninvasive cancer detection. Nanomedicine: Nanotechnology, Biology and Medicine. 2011;7(5):655-63.
24. Cao H, Liu X. Silver nanoparticles-modified films versus biomedical device-associated infections. Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology. 2010;2(6):670-84.
25. Walser T, Demou E, Lang DJ, Hellweg S. Prospective environmental life cycle assessment of nanosilver T-shirts. Environmental science & technology. 2011;45(10):4570-8.
26. Benn TM, Westerhoff P. Nanoparticle silver released into water from commercially available sock fabrics. Environmental science & technology. 2008;42(11):4133-9.
27. Cushen M, Kerry J, Morris M, Cruz-Romero M, Cummins E. Nanotechnologies in the food industry–Recent developments, risks and regulation. Trends in food science & technology. 2012 ;24(1):30-46.
28. Huang Y, Chen S, Bing X, Gao C, Wang T, Yuan B. Nanosilver migrated into food-simulating solutions from commercially available food fresh containers. Packaging Technology and Science. 2011;24(5):291-7.
29. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology advances. 2009;27(1):76-83.
30. Matsumura Y, Yoshikata K, Kunisaki SI, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Applied and environmental microbiology. 2003;69(7):4278-81.
31. Gupta A, Maynes M, Silver S. Effects of halides on plasmid-mediated silver resistance in Escherichia coli. Applied and environmental microbiology. 1998;64(12):5042-5.

32. Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. Journal of biomedical materials research. 2000;52(4):662-8.
33. Nover L, Scharf KD, Neumann D. Formation of cytoplasmic heat shock granules in tomato cell cultures and leaves. Molecular and cellular biology. 1983;3(9):1648-55.
34. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. 2004;275(1):177-82.
35. Zhang XF. Zhi-Guo liu, Wei shen, Sangiliyandi Gurunathan. SilverNanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. International Journal of Molecular Sciences. 2016;17:1534.
36. Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterialaction of silver ions. Letters in applied microbiology. 1997;25(4):279-83.
37. Touchon M, Hoede C, Tenaillon O, Barbe V, Baeriswyl S, Bidet P, Bingen E, Bonacorsi S, Bouchier C, Bouvet O, Calteau A. Organised genome dynamics in the Escherichia coli species results in highly diverse adaptive paths. PLoS genetics. 2009;5(1):e1000344.
38. Kakkar Thukral D, Dumoga S, K Mishra A. Solid lipid nanoparticles: promising therapeutic nanocarriers for drug delivery. Current drug delivery. 2014;11(6):771-91.
39. Kaper, James B., James P. Nataro, and Harry LT Mobley. "Pathogenic escherichia coli." *Nature reviews microbiology* 2.2 (2004): 123-140.
40. Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, Griffin PM. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerging infectious diseases. 2013;19(3):407.
41. Lupo A, Coyne S, Berendonk TU. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Frontiers in microbiology. 2012, 26;3:18.
42. WHO Scientific Group on Health Aspects of Use of Treated Wastewater for Agriculture and Aquaculture. Health guidelines for the use of wastewater in agriculture and aquaculture: report of a WHO Scientific group. World Health Organization; 1989.
43. Holmes S. South African water quality guidelines. Volume 7: aquatic ecosystems.
44. Foppen JW, Schijven JF. Evaluation of data from the literature on the transport and survival of Escherichia coli and thermotolerant coliforms in aquifers under saturate Ishii S, Sadowsky MJ. Escherichia coli in the environment: implications for water quality and human health. Microbes and environments. 2008;23(2):101-8.d conditions. Water Research. 2006;40(3):401-26.
45. Ishii S, Sadowsky MJ. Escherichia coli in the environment: implications for water quality and human health. Microbes and environments. 2008;23(2):101-8.
46. Fremaux B, Prigent-Combaret C, Vernozy-Rozand C. Long-term survival of Shiga toxin-producing Escherichia coli in cattle effluents and environment: an updated review. Veterinary microbiology. 2008;132(1-2):1-8.
47. Topp E, Welsh M, Tien YC, Dang A, Lazarovits G, Conn K, Zhu H. Strain-dependent variability in growth and survival of Escherichia coli in agricultural soil. FEMS microbiology ecology. 2003 ;44(3):303-8.
48. Ibekwe AM, Murinda SE, Graves AK. Genetic diversity and antimicrobial resistance of Escherichia coli from human and animal sources uncovers multiple resistances from human sources. PLoS One. 2011;6(6):e20819.
49. Janezic KJ, Ferry B, Hendricks EW, Janiga BA, Johnson T, Murphy S, Roberts ME, Scott SM, Theisen AN, Hung KF, Daniel SL. Phenotypic and genotypic characterization of Escherichia coli isolated from untreated surface waters. The open microbiology journal. 2013;7:9.
50. Russell B. Quantitative Study of the Antimicrobial Effects of Silver on the Motility of Escherichia coli. University of Arkansas; 2019.

51. Loo YY, Rukayadi Y, Nor-Khaizura MA, Kuan CH, Chieng BW, Nishibuchi M, Radu S. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Frontiers in microbiology. 2018 ;9:1555.
52. Paredes D, Ortiz C, Torres R. Synthesis, characterization, and evaluation of antibacterial effect of Ag nanoparticles against Escherichia coli O157: H7 and methicillin-resistant Staphylococcus aureus (MRSA). International journal of nanomedicine. 2014;9:1717.
53. Kar D, Bandyopadhyay S, Dimri U, Mondal DB, Nanda PK, Das AK, Batabyal S, Dandapat P, Bandyopadhyay S. Antibacterial effect of silver nanoparticles and capsaicin against MDR-ESBL producing Escherichia coli: an in vitro study. Asian Pacific Journal of Tropical Disease. 2016;6(10):807-10.
54. Ansari MA, Khan HM, Khan AA, Ahmad MK, Mahdi AA, Pal R, Cameotra SS. Interaction of silver nanoparticles with Escherichia coli and their cell envelope biomolecules. Journal of basic microbiology. 2014;54(9):905-15.
55. Prieto EI, Kiat AA. The antimicrobial action of silver nanoparticles on Escherichia coli as revealed by atomic force microscopy. Philipp Sci Lett. 2017;10:123-9.
56. Raffi M, Hussain F, Bhatti TM, Akhter JI, Hameed A, Hasan MM. Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224. Journal of materials science and technology. 2008 ;24(2):192-6.
57. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, biology and medicine. 2007 ;3(1):95-101.
58. Le AT, Huy PT, Tam PD, Huy TQ, Cam PD, Kudrinskiy AA, Krutyakov YA. Green synthesis of finely-dispersed highly bactericidal silver nanoparticles via modified Tollens technique. Current Applied Physics. 2010;10(3):910-6.
59. Parameswari E, Udayasoorian C, Sebastian SP, Jayabalakrishnan RM. The bactericidal potential of silver nanoparticles. International Research Journal of Biotechnology. 2010;1(3):044-9.
60. Thanh NV, Phong NT. Investigation of antibacterial activity of cotton fabric incorporating nano silver colloid. InJournal of Physics: Conference Series 2009 (Vol. 187, No. 1, p. 012072). IOP Publishing.
61. Shavandi Z, Ghazanfari T, NAZARI MK, ABDI A. The inhibitory effect of colloidal silver nanoparticles on three bacterial strains and macrophages in a 24-hrs cell culture.
62. Ghosh S, Kaushik R, Nagalakshmi K, Hoti SL, Menezes GA, Harish BN, Vasan HN. Antimicrobial activity of highly stable silver nanoparticles embedded in agar–agar matrix as a thin film. Carbohydrate Research. 2010 ;345(15):2220-7.
63. Tiwari DK, Behari J. Biocidal nature of combined treatment of Ag-nanoparticle and ultrasonic irradiation in Escherichia coli DH5. Advances in Biological Research. 2009;3(3-4):89-95.
64. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, biology and medicine. 2007 ;3(1):95-101.
65. Raza MA, Kanwal Z, Rauf A, Sabri AN, Riaz S, Naseem S. Size-and shape-dependent antibacterial studies of silver nanoparticles synthesized by wet chemical routes. Nanomaterials. 2016 ;6(4):74.
66. Cunha FA, Maia KR, Mallman EJ, CUNHA MD, Maciel AA, SOUZA IP, Menezes EA, Fechine PB. Silver nanoparticles-disk diffusion test against Escherichia coli isolates. Revista do Instituto de Medicina Tropical de São Paulo. 2016; 22;58.
67. Rao P, Chandraprasad MS, Lakshmi YN, Rao J, Aishwarya P, Shetty S. Biosynthesis of silver nanoparticles using lemon extract and its antibacterial activity. International Journal of Multidisciplinary and Current Research. 2014;2:165-9.