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Study the Effect of Antibiotic of Gentamicin Coated with Silver Nanoparticles (AgNPs) on *Pseudomonas aeruginosa* Isolated from Burns

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ABSTRACT

The study sample was microscopic and biochemical, and 20 samples were isolated from the columns and biochemistry, and 20 samples were isolated from the samples and biochemistry. The aim of this study was to evaluate the antimicrobial activity of the combination antibiotic (gentamicin) coated with silver nanoparticles against *Pseudomonas aeruginosa* isolated from burns. The combination of gentamicin and silver nanoparticles resulted in a synergistic effect against antibiotic-resistant *Pseudomonas aeruginosa* isolates.

INTRODUCTION

Pseudomonas aeruginosa is widespread in nature and is commonly found in moist environments and in hospitals. It is known to cause disease in people with compromised, altered and reduced defenses eg, neutropenia, chemotherapy, wounds and burns (Riedel *et al*., 2019). *Pseudomonas aeruginosa* possesses several factors that play an important role in the pathogenesis of disease. These factors include biofilm formation, protein secretion system, iron acquisition system, quorum sensing, and others (Marshall *et al*., 2017). Increased resistance to antimicrobial agents is a major public health problem worldwide (Daya *et al*., 2015). One of the most promising strategies for overcoming microbial resistance is the use of nanoparticles (Cioffi *et al*., 2005). Encapsulated nano-antibiotics are seen as a good alternative to improve existing therapies and represent a promising strategy to overcome the mucus barrier and prolong drug retention in lung cells as previously reported by researchers (Poyner *et al*., 1995). For antibacterial properties, gentamicin CN was loaded on silver nanoparticles AgNPs and used in wound dressings for the purpose of treating infection (Bie *et al*., 2020). Aminoglycoside antibiotics are widely used to treat various types of bacterial infections due to their broad spectrum of activity. The spectrum includes Gram-negative bacteria (*Pseudomonas aeruginosa*). However, aminoglycoside resistance can occur, which has led to the search for various combinations of these antibiotics with other antimicrobial agents (Rodrigues *et al*., 2009).

The aim of the study is a comparison between the effect of the antibiotic gentamicin coated with silver nanoparticles and the antibiotic alone on the activity of *Pseudomonas aeruginosa.*

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MATERIALS AND METHODS

Clinical samples were collected from burn patients using cotton swabs. The samples were cultured and diagnosed under a light microscope. Cultivation characteristics, including the growth of colonies on different media (nutrient agar, blood agar, McConkey agar, Cetrimide agar). The growing bacteria were characterized in terms of shape, color and dissolution pattern. Blood and lactose fermentation, biochemical tests were also conducted to check the properties of the isolate bacteria, and these tests included the indole test to verify the production of indole. The methyl red test to check the fermentation sugar and acid production. The Vogus-Proscauer test to detect the acetone compound, the citrate test to verify the consumption of citrate as a single carbon source and formation of sodium carbonate. Urease test indicating hydrolysis to form urea and ammonium, oxidase test to verify production of cytochrome c, catalase and fermentation tests for sugars (Brown and Smith, 2017).

Preparation of the Plant Extract:

About 20 gm of coriander leaves were taken, washed well four times with de-ionized water to remove dust particles, and dried in the air at room temperature, then the leaves were ground into a fine powder and added to 100 ml of de-ionized water, and left for 20 minutes to boil. At 60ºC, after boiling, the leaf extract was cooled at room temperatures, filtration. 75 ml of the yellow-transparent leaf extract was taken, which was stored at 4ºC in the refrigerator (Rhamah *et al*., 2021).

Preparation of Silver Nanoparticles:

Silver nanoparticles were prepared by dissolving 0.067 g of AgNO3 in 100 ml of deionized water. A 4 mM AgNO3 solution was made, stored in the dark to prevent oxidation, and used to prepare AgNPs. After that, 5 ml of coriander leaf extract was added to 45 ml of a solution. Silver nitrate, AgNO3, was placed on a heat plate device and a magnetic stirrer was used for an hour. After that, the color of the reaction mixture changed from transparent yellow to dark brown, indicating the formation of silver nanoparticles AgNPs. The AgNPs solution was collecting and placing in the centrifuge. For the purpose of sedimentation, the excess liquid was removed and the precipitate was taken by drying it in an electric oven at a temperature of 40ºC until it was completely dry and we obtained nano powder of silver (Khan *et al*., 2018).

Antibiotic Loading on Silver Nanoparticles:

0.02 g of silver nanoparticles were dissolved in 100 mL distilled water using a magnetic stirrer rod at 1000 (rpm) for 30 min. 0.2 g of antifreeze powder was dissolved in 100 mL distilled water using a magnetic stirrer for 15 min, then mixed 25 ml of each of the two solutions by magnetic stirrer for 45 minutes, after homogenization the mixture was placed in the ultrasonic device for 45 minutes, in order to obtain the smallest possible volume. The solution was filtered using 0.22-volume Whatman filter paper (Ibraheem *et al*., 2022).

Diagnostic Techniques of Prepared Silver Nanoparticles:

Silver nanoparticles have been extensively investigated using visible and ultraviolet light spectroscopy. Fourier transform infrared (FT-IR) spectroscopy, and energy-dispersive X-ray silver nanoparticles (EDX), while the properties were evaluated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).Studying the inhibitory activity of silver nanoparticles, the antibiotic alone, and the antibiotic coated with Pseudomonas aeruginosa (inhibition zone diameter).

The Agar diffusion method was used by drilling wells on Muller Hinton Agar with a cork bore to make holes with equal dimensions to prevent overlapping of the diameters of inhibition and a diameter of

6 mm to contain nanoparticle solutions of 60 microliters per hole after spreading 0.1 ml of the bacterial suspension on the medium for testing. The sensitivity of bacteria to nanomaterials, and concentrations (64,32,16,8,4) µg/ ml of silver nanoparticles and the antibiotic gentamicin were used, then the dishes were left in the refrigerator for one hour to spread the silver nano solutions, and then the dishes were incubated at 37 °C for 24 hours. The results were read using a millimeter scale to measure the diameter of the inhibition zone (Al-Hamdany *et al*., 2021).

Statistics Analysis:

The results were analyzed statistically by applying the ANOVA test, complete random design (CRD), and the arithmetic means were compared with the tekken multinomial test with a probability level of 0.05% (SAS, 2012).

RESULTS AND DISCUSSION

Bacterial swabs from burn infections of different ages of both sexes were collected for the period between October 2021/ February 2022 from the Burn Hospital in the Medical City in Baghdad and private clinics in Samarra. After conducting morphological and biochemical tests, 20 samples were obtained. *Pseudomonas aeruginosa* was also diagnosed by studying the phenotypic characteristics of the bacteria by cultivating it on MacConkey medium, as the colonies appeared in a pale color, due to its inability to ferment the sugar lactose (Forbes *et al*., 2007). As for solid blood medium, the colonies showed their ability to blood analysis of the beta type, which is evidence of the ability of the bacteria to produce the enzyme hemolysin (Selim *et al*., 2015). It cultivated on the medium of solid cetrimide, as the colonies appeared in a greenish-yellow color, which is called the pyoverdine dye, or in a greenish-blue color, which is called the pyocyanin dye, which fluoresces when exposed to ultraviolet light. These dyes are distinguished by their being dissolved in water (Sudhakar *et al*., 2015).

AgNPs Biosynthesis:

Coriander leaf extract was used as a reducing agent and stabilizer in the biosynthesis of silver nanoparticles. The results of the study showed the appearance of a precipitate at the bottom, and this is evidence of the process of synthesis of silver nanoparticles. One of the most important reasons for using silver nanoparticles is biosynthesis. Cheap price, safe for the environment, risk-free, easy to operate, and low toxicity.

Characterization of AgNPs:

1. UV Analysis and Visible Spectrophotometer (UV-Vis):

The formation of green AgNPs synthesis was demonstrated by changing the visible color (colorless to dark brown) after completion of the reaction between coriander plant extract and silver nitrate. The optical properties of the NPs were studied using UV- analysis visible and spectrometer. The absorbance of the silver nanoparticles AgNPs was in the range spectrophotometers from 200 to 900 nm and the resulting solution showed a constant λmax maximum at 454nm, confirming the ordered size and shape of AgNPs (Pinzaru *et al*., 2018).

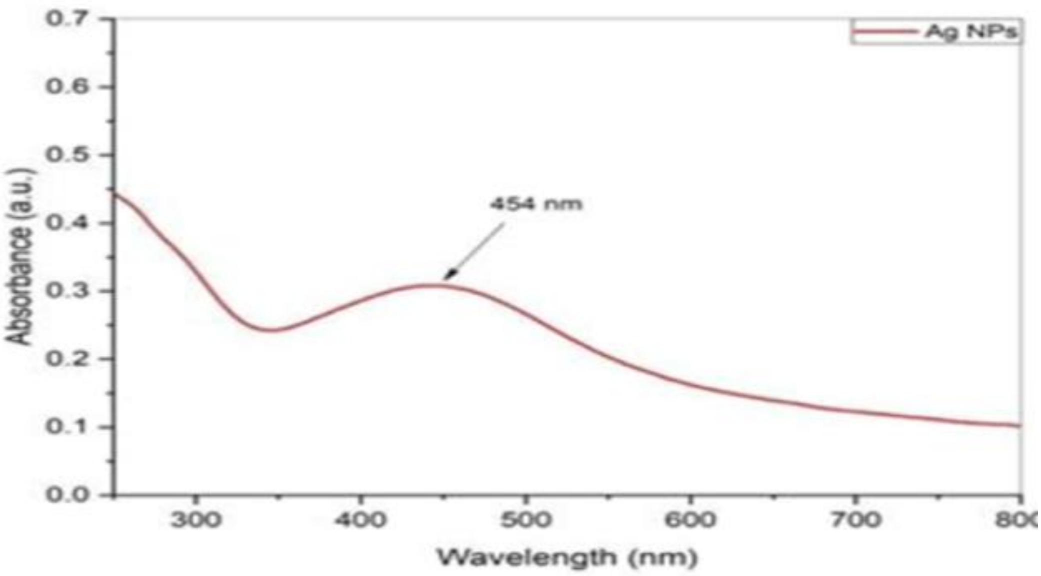

Fig. 1: UV-visible spectrum of AgNPs.

2. Infrared Spectroscopy (FTIR):

The AgNPs samples were examined by FT-IR to determine the presence of encapsulating particles, as well as the effective stability of the synthesized metal NPs. FTIR analysis of silver AgNPs nanoparticles showed the presence of transmittance peaks at 3435.56, 2354.57, 2080.76, 1958.82, 1635.80, 1455.50, 1046.57 and 667.44 cm-1 (Muzamil *et al.*, 2014).

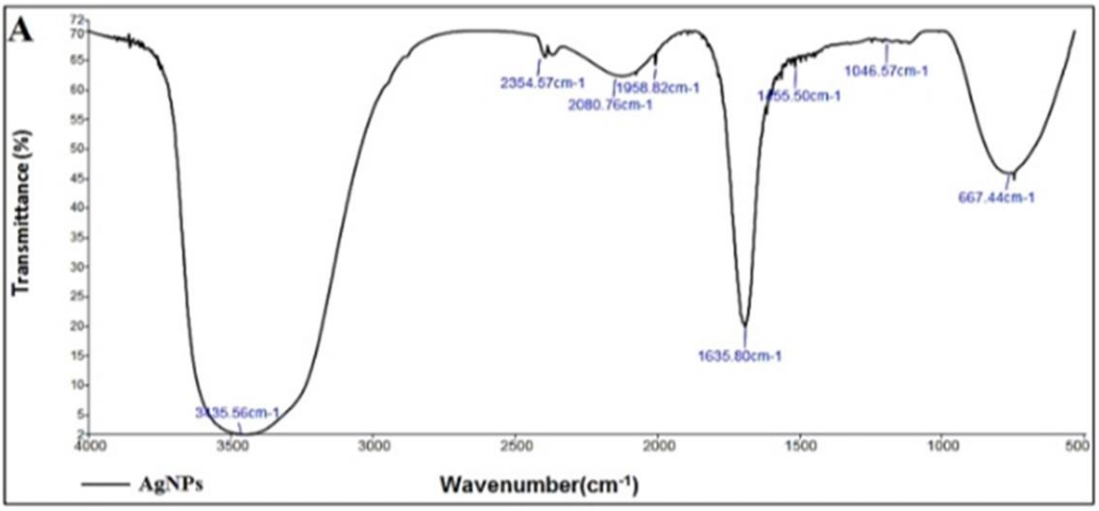

Fig. 2: FTIR spectroscopy of AgNPs.

3. X-ray diffraction (XRD) Analysis:

The XRD pattern of AgNPs at 2θ showed four peaks, 32.12º, 38.04º, 46.21º, and 64.18º, corresponding to (101), (111), (200), and (220), respectively.

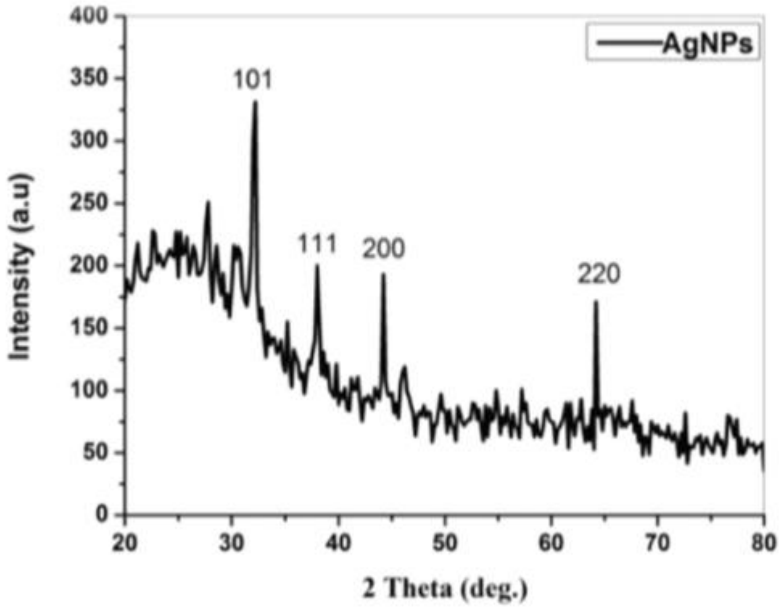

Fig. 3: X-ray diffraction (XRD) of AgNPs**.**

4. Field Emission Scanning Electron Microscopy (FE-SEM) and Energy-Dispersive X-Ray Analysis EDX:

The SEM image of the as-synthesized high-density green AgNPs silver nanoparticles confirmed the evolution of the silver nanostructures. The SEM micrographs of the NPs obtained in the filter showed that the AgNPs were spherical in shape and well distributed in the solution without aggregation. Most of the NPs were spherical in shape, and the AgNPs had a smooth surface. The dimensions were from 32.08 nm to 43.28 nm (Zhang *et al.*, 2016).

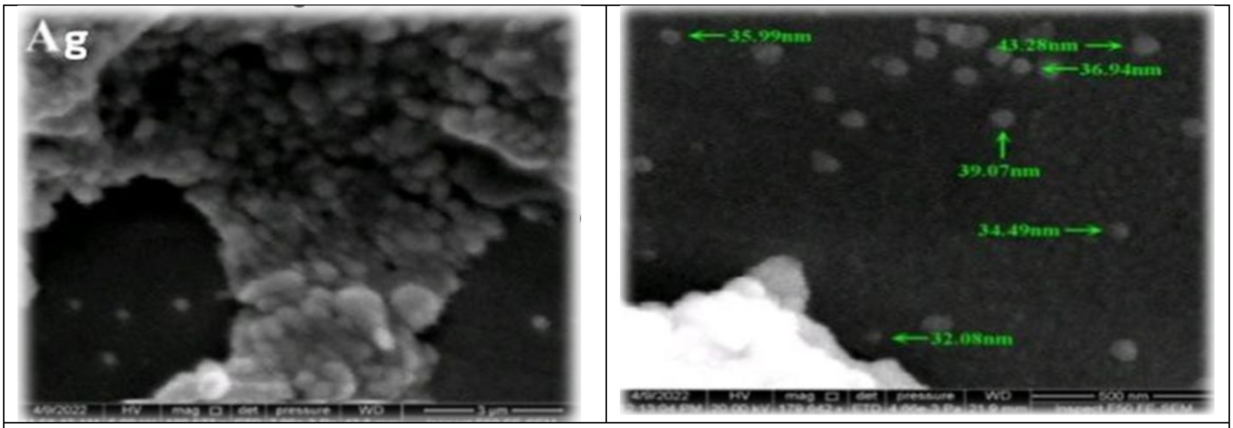

Fig. 4: FE-SEM scanning electron microscopy of AgNPs.

Energy dispersive X-ray analysis (EDX) showed that the weight percentage of Ag in AgNPs was 60.4% of the total constituents of the sample, with only small proportions of carbon (C), oxygen (O), sulfur (S), and the presence of sodium (Na) . They are component parts of the chemicals used in the synthesis of AgNPs. The table showed that there were significant differences between the inhibitory effects of all concentrations of AgNPS nanoparticles.

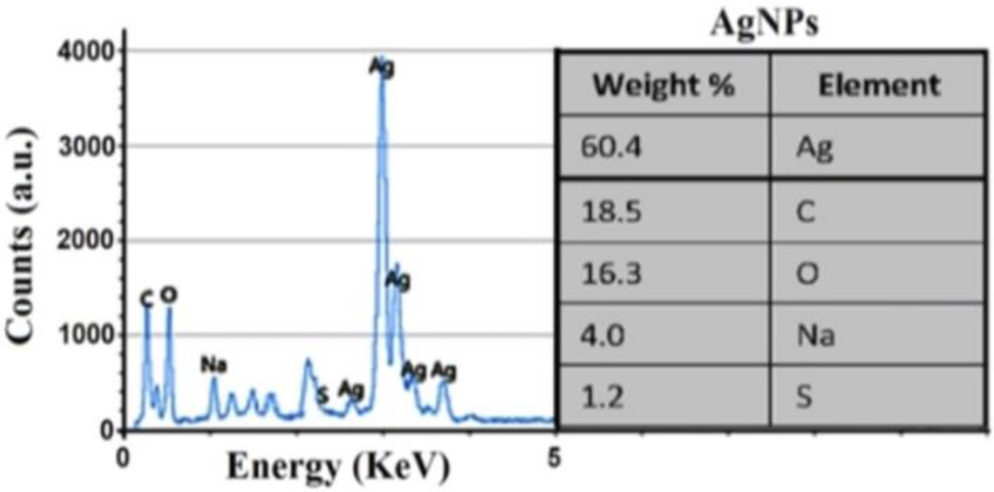

Fig. 5: Energy dispersive X-ray analysis EDX.

6. TEM Scanning Electron Microscopy:

To analyze the morphology and size distributions of silver AgNPs, TEM analysis was performed using a 77.500 kx magnification. The results of the enlarged image showed that the majority of NPs have spherical shapes with different dimensions.

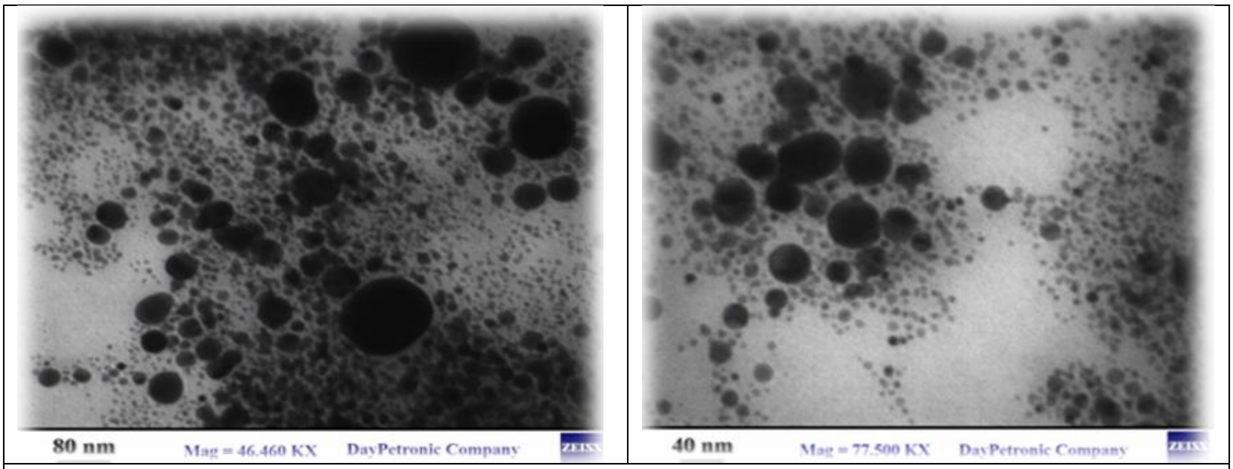

Fig. 6: AgNPs under TEM electron microscopy.

Determination of the Effectiveness of AgNPs, Anti-Gentamicin Alone, and Anti-Gentamicin Coated with Silver Nanoparticles on the Growth of Bacterial Isolates Using Agar Plate Method (inhibition diameter) mm:

The results of using the agar diffusion method to evaluate the effect of silver nanoparticles AgNPs used in this study showed that five concentrations (64, 32, 16, 8, 4)µg/ml were used. The results showed that the inhibition rate of silver nanoparticles AgNPs on the growth of *Pseudomonas aeruginosa* was 19.6 mm at a concentration of 64µg/ml, and did not give inhibition at concentrations between 8 and 4 µg/ml.

The inhibition rate of anti-gentamicin was 13.3 mm at a concentration of 64 micrograms/ml, while the rate of inhibition of anti-gentamicin coated with silver nanoparticles was 16 mm at a concentration of 64 µg/ml.

The results showed that there were significant differences at the probability level of $P<0.05$, as shown in the Table (1).

Table 1: Inhibitory activity.

Average active ingredient	µg/ml concentration					Effective Material
	4	8	16	32	64	
10.50 A	0	0	14.3	18.6	19.6	**AgNPs**
B 4.98	0	0	0	10.5	14.7	**CN**
C6.14	0	0	0	14.7	16	**CN- AgNPs**

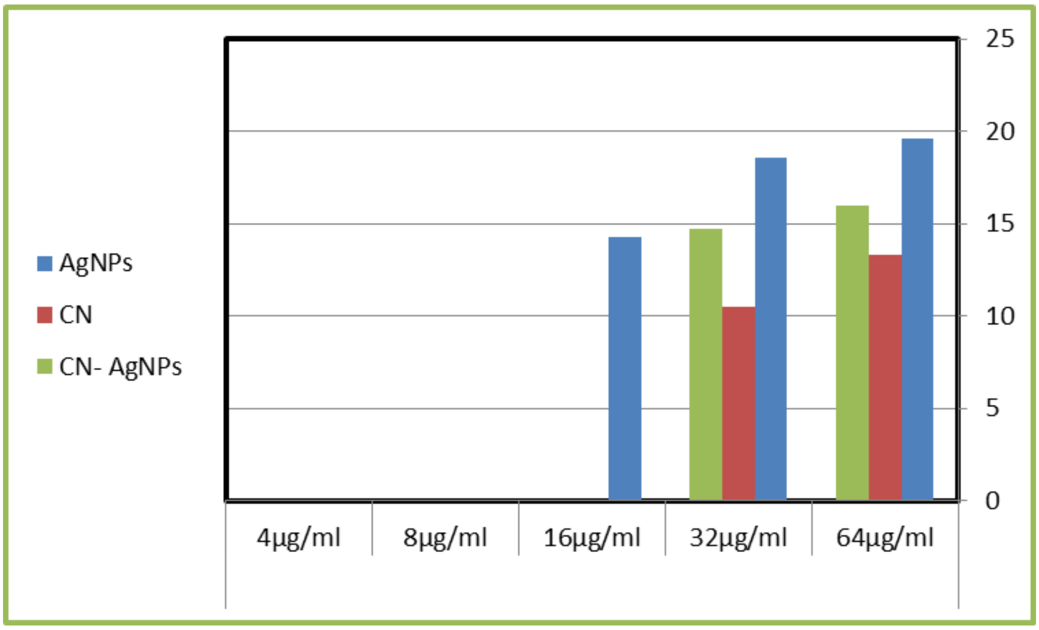

Fig. 7: Inhibitory activity.

The antibacterial activity of these AgNPs may be attributed to the generation of oxidative stress and disruption of DNA replication or AgNPs can directly cause bacterial cell lysis by damaging cell membranes (da Silva *et al*.,2013). The antibacterial effects of nanoparticles could be Silver. Also, results from the interaction of the nanoparticles with putative peptides that are essential for cell survival and division.

Silver nanoparticles found that in the initial phase of the reaction, they attach to the bacterial cell wall, after which they enter the bacterium and kill the bacterial cell by destroying the membrane. The results showed the potential for using AgNPs as an alternative to the conventional antimicrobial agents currently in use (Salomoni *et al*., 2017). There are reports describing AgNPs-mediated DNA damage due to the ingress of Ag+ ions between purine and pyrimidine base pairs. This event leads to the breakdown of the DNA double-helical structure followed by the phenomenon of disrupted replication (Pramanik *et al*., 2016). The mechanism of cell death induced by nanosilver is that silver may disrupt several bacterial cellular processes, including disulfide bond formation, and metabolism. These changes may lead to increased production of reactive oxygen (ROS) and increased food permeability that can stimulate group activity. A wide range of antibiotics for Gram-negative bacteria in different metabolic states (Fayaz *et al*., 2010).

These results were in agreement with a study conducted on the synergy of silver nanoparticles, which confirmed the presence of synergistic activity of silver nanoparticles with antibiotics, against *P. aeruginosa*. The report concluded that the synergistic action of the antimicrobial agent can significantly reduce the side effects of antibiotics by reducing the doses and thus the use of nanoparticles with antibiotics can improve their effectiveness against different pathogenic resistant microbes (Ruparelia *et al*., 2008).

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