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Some Nanoparticles Effects on *Proteus sp.* and *Klebsiella* Sp. Isolated from Water

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Abstract The purpose of the present study was to elucidate the antimicrobial activity and mechanism of silver, platinum, palladium and copper nanoparticles on *Proteus sp.* and *Klebsiella* Sp. isolated from polluted water samples. This study investigated the growth of the two tested bacterial cells in order to observe the action of different concentrations of nanoparticles in addition to the effects on the morphological structure and nucleic acid of these bacteria, It showed AgNPs exhibited a potentially antibacterial effects at most investigated concentrations, While, no detection for the antibacterial activity of PtNPs, PdNPs or CuNPs against both bacterial isolates at all tested concentrations was investigated. The cell morphology of normal and AgNPs-treated bacteria cells was assessed by transmission electron microscopy (TEM), there were investigated a randomly distributed nuclear area and obvious bright electron areas in the center of bacterial cells, it was also noticed that, a rupture in the cell wall of both treated bacterial cells. The effects of silver nanoparticles on genome of bacterial cells revealed that there was a destructive effect on *Proteus* and *Klebsiella* genome.

Keywords: *metal nanoparticles, antibacterial activity, action mechanism, Proteus sp., Klebsiella sp.*

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1. Introduction

Nanotechnology is a control of matter nanometer scale to develop new solutions involves the manipulation of matter on a near atomic scale to produce new structure, devices, and materials [1]. Nanoparticles usually ranging in dimension from 1-100 nanometers (nm) have properties unique from their bulk equivalent. With the decrease in the dimensions of the materials to the atomic level, their properties change and they possess unique physico-chemical, optical and biological properties which can be manipulated suitably for desired applications [2]. Nanosilver has become one of the most popular nanoparticles due to its many applications and relatively low manufacturing costs. It is currently being used for a wide variety of commercial products including medical applications, water purification, antimicrobial uses, paints, coatings, food packaging. Impregnating other materials with silver nanoparticles is a practical way to exploit the germ fighting properties of silver [3].

Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio). It is suggested that these may be used in future at large scale water purification [2]. Silver is a safe and effective anti-bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria such as *Escherchia coli* (*E. coli*) and *Staphylococcus aureas*, *Staphylococcus epidermidis [4]*. Metal nanoparticles with antimicrobial activity when embedded and coated to surfaces can find immense applications in water treatment, synthetic textiles, biomedical and surgical devices, food processing and packaging [5].

Proteus is a genus of Gram –negative proteobacteria. *Proteus* bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, in sewage, in manure soil, and in human and animal feces. They are opportunistic pathogens, commonly responsible for urinary and septic infections. Three species, *P. vulgaris*, *P. mirabilis and P. penneri* are opportunistic human pathogens. *Proteus* includes pathogens responsible for many human urinary tract infections. *P. mirabilis* causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins. *P. vulgaris* is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immunosuppressed individuals. *P. vulgaris* occurs naturally in the intestine of humans and a wide variety of animals, and in manure, soil, and polluted waters. *P. mirabilis*, once attached to the urinary tract, infects the kidney more commonly than *E. coli*. *P. mirabilis* is often found as a free-living organism in soil and water. About 10-15% of kidney stones are caused by alkalinization of the urine by the action of the urease enzyme (which splits urea into ammonia and carbon dioxide) of *Proteus* (and other) bacterial spp. [6,7].

Klebsiella spp. have been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), urinary tract infection (6 to 17%), wound infections (2 to 4%), intensive care unit infections (4 to 17%), and neonatal septicaemias (3 to 20%). *Klebsiella* spp. can also cause hepatic infections, and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, fascial space infections of the head and neck, and septic arthritis [8].

In the present investigation, it will be evaluated the antibacterial effect of silver, platinum, palladium and copper nanoparticles on two pathogenic bacteria isolates, *Proteus sp.* and *Klebsiella* sp. that were isolated from microbial polluted water samples and it will be demonstrated the nature of interaction between the potentially effective metal nanoparticles and the tested bacteria.

2. Materials and Methods

2.1. Metallic Nanoparticles

Four different types, silver (AgNPs), Platinum (PtNPs), Palladium (PdNPs) and copper (CuNPs) nanoparticles were provided by Chemistry Department, Science faculty, Taif university, KSA. TEM images of single metals nanoparticles showed that the particles AgNPs, PtNPs, PdNPs and CuNPs on NaY zeolite were spherical with particle size of ≈ 38; 19; 34 and 68 nm, respectively (Figure 1). Metal nanoparticles were diluted at different concentrations of (i.e. 0, 0.5, 1.0, 10, 20, 25 and 100 mg/l) using distilled water. All solutions were stored at 4^{o}C until further use.

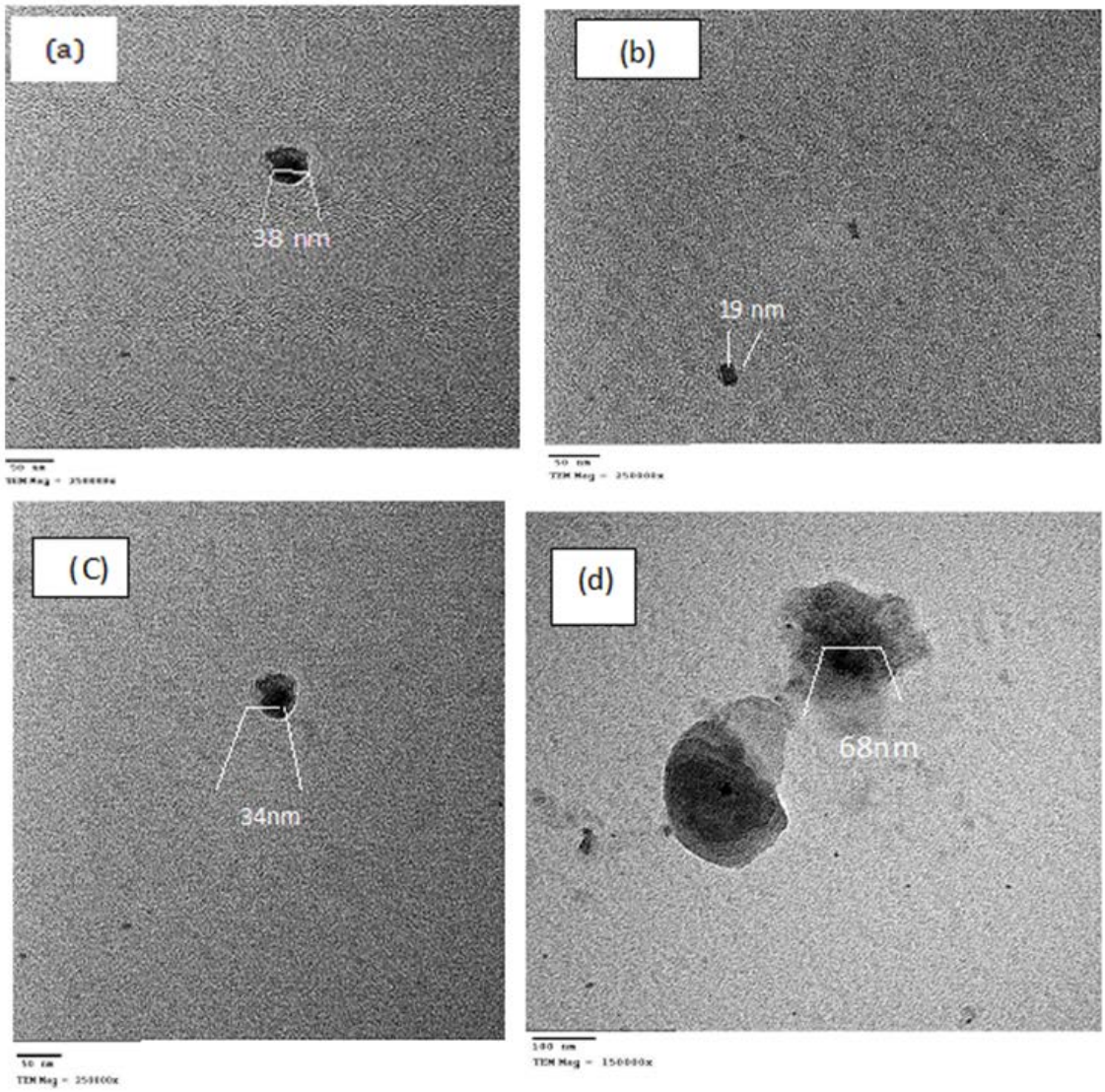

Figure 1. TEM image of: (a) AgNPs (b) PtNPs samples (c) PdNPs (d) CuNPs

2.2. Bacterial Isolation and Identification

Proteus sp. and *Klebsiella* Sp. two bacterial isolates that were isolated from microbial polluted water samples that were collected from a reservoir of water storage, Alkhurmah City, KSA using a selective mac-Conkey agar media. *Proteus sp.* was selected as gram negative non-lactose fermenting bacterial isolate and *Klebsiella* sp. was selected as gram negative lactose fermenting bacterial isolate. These obtained bacterial isolates were more identified to genus level using Catalase reaction, indole test, H_2S production [9].

2.3. Antibacterial Activity of Nanoparticles

2.3.1. Disc Diffusion Method

The modified disc diffusion method was used to evaluate the antimicrobial activity of AgNPs, PtNPs, PdNPs and CuNPs against the two tested bacterial isolates. This method was performed Muller Hinton agar media [4]. 6 mm sterile paper discs impregnated with 1, 10, 20, 25, 50 and 100 mg/L of silver, palladium, platinum and copper nanoparticles, separately. Nanoparticles samples was placed on inoculated Muller Hinton agar plate, which were then incubated for 24 h at 37ºC. Inhibition zone was monitored after incubation, the presence of bacterial growth inhibition halo around the samples were measured in millimeters.

2.3.2. Transmission Electron Microscopy

The interaction between bacterial isolates and the potentially effective AgNPs was examined by transmission electron microscopy TEM. Muller Hinton broth medium (10 mL), AgNPs and bacteria were added to a 20 mL test tube to obtain the final concentrations of 10 mg/L AgNPs and 10^6 CFU/mL bacteria cells. Control group was generated without AgNPs. The test tubes were incubated at 37°C ± 2°C and shaken at 150 rpm for 12 hours, after which the cultures were centrifuged and the pellets harvested for morphology and structure analysis by TEM according to the one described by [10]. The examination was carried out by (Hitachi H-7650; Pleasanton, CA) at Center for Mycology and The Regional Biotechnology, Al-Azhar University, Cairo, Egypt.

2.3.3. AgNPs Effects on the DNA of Bacteria

To determine AgNPs effect on the DNA damage of the treated bacterial isolates, *Proteus sp.* and *Klebsiella* sp., The reaction mixture containing 0.5 mL LB broth medium. AgNPs, 10mg/l and bacteria in 20 mM potassium phosphate buffer (pH 7.4) was pre-incubated for 24 h at 37°C [11]. The amount of DNA from normal bacteria cells and treated bacteria cells by AgNPs was evaluated by agarose gel electrophoresis. 1×10^6 cells were lysed in 250 µL cell lysis buffer containing 50 mM Tris HCl, pH 8.0, 10 mM ethylene diamine tetraacetic acid, 0.1 M NaCl, and 0.5% sodium dodecyl sulfate. The lysate was incubated with 0.5 mg/mL *RNase* A at 37°C for one hour, and then with 0.2 mg/mL *proteinase* K at 50°C overnight. *Phenol* extraction of this mixture was carried out, and DNA in the aqueous phase was precipitated by 25 µL (1/10 volume) of 7.5 M *ammonium acetate* and 250 µL (1/1 volume) *isopropanol*. DNA electrophoresis was performed in a 1% agarose gel containing 1µg/mL ethidium bromide at 70 V, and the DNA fragments were visualized by exposing the gel to ultraviolet light, followed by photography[12].

3. Results

3.1. Antibacterial Activity

3.1.1. Agar Disks Diffusion Test

The antibacterial effect of the prepared AgNPs, PtNPs, PdNPs and CuNPs nanoparticles at different concentrations was studied on two bacterial isolates, *Proteus sp.* and *Klebsiella.* sp. Table 1 & Table 2 and Figure 2 showed the inhibition zone of different concentrations of metals nanoparticles. Results showed that, AgNPs exhibited inhibition zone (mm) of about 0, 0, 18, 19.5, 20, 24 and 24.5 mm in diameter for *Klebsiella* sp. and inhibition zone 0, 11, 15, 16, 18.5 and 20 mm for *Proteus sp.* at 0.5, 1.0, 20, 25, 50 and 100 mg/L of silver nanoparticles concentrations, respectively. While, no detection for the antibacterial activity of PtNPs, PdNPs or CuNPs against both bacterial isolates at all tested concentrations.

Table 1. Zone of inhibition (mm) of metal nanoparticles against *Proteus sp.*

Metal nanoparticles	Nanoparticles concentration (mg/L)						
	0.5	**1.0**	**10**	**20**	**25**	**50**	**100**
AgNPs	**0.0**	**0.0**	**11.0 ± 1.0**	**15.0 ± 1.0**	**16.0 ± 0.0**	**18.5 ± 1.2**	**20.0 ± 1.0**
PtNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
PdNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
CuNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

Table 2. Zone of inhibition (mm) of metal nanoparticles against *Klebsiella sp.*

Metal nanoparticles	Nanoparticles concentration (mg/L)						
	0.5	**1.0**	**10**	**20**	**25**	**50**	**100**
AgNPs	**0.0**	**0.0**	**18.0 ± 1.0**	**19.5 ± 1.0**	**20.0 ± 1.0**	**24.0 ± 1.0**	**24.5 ± 1.0**
PtNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
PdNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
CuNPs	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**

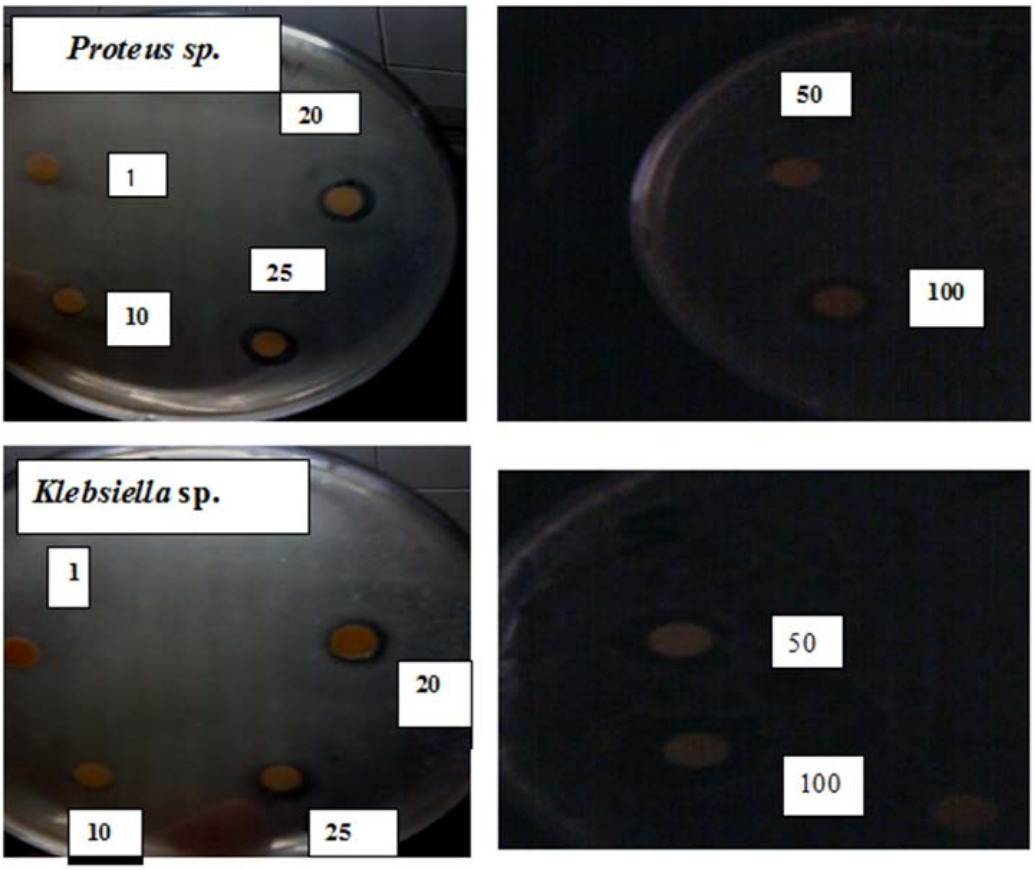

Figure 2. The diameter of inhibition zone surrounding different concentrations of silver nanoparticle impregnated disks against *Proteus sp.* and *Klebsiella* sp.

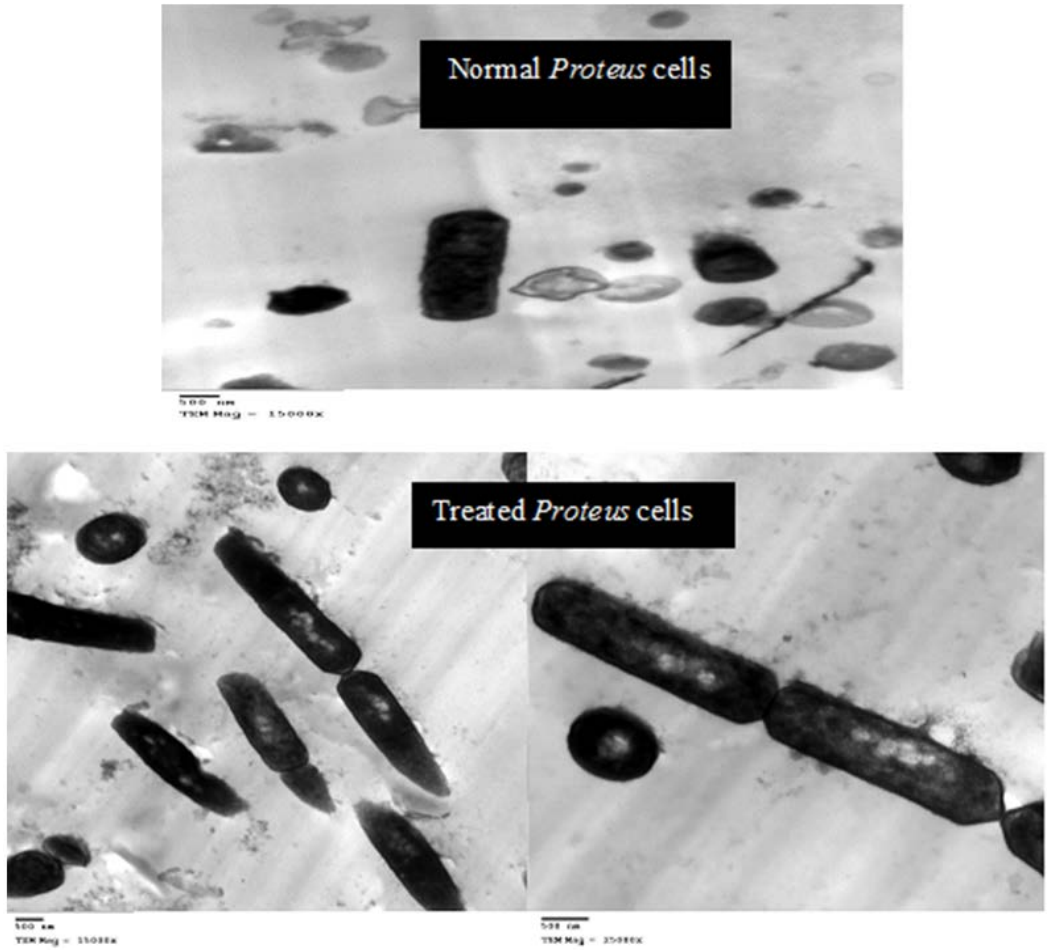

Figure 3. Structure of normal and AgNPs treated *Proteus sp.* cells under transmission electron microscope (TEM)

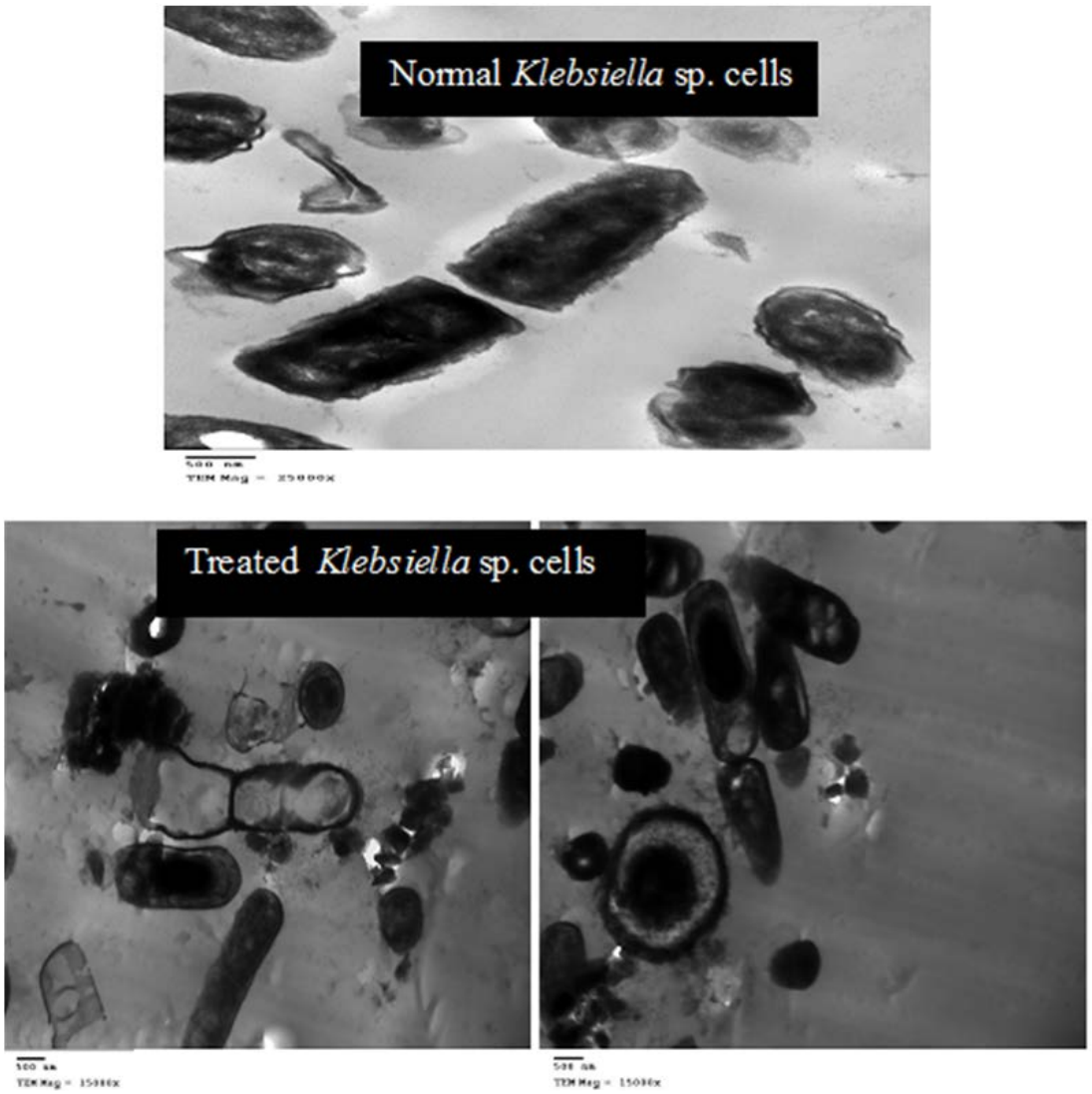

Figure 4. Structure of normal and AgNPs-treated *Kelbsiella* sp. cells under transmission electron micrope (TEM)

3.1.2. Transmission Electron Microscopy

Morphology and structure of normal *Proteus* and *Klebsiella* cells and treated cells by AgNPs at (10 mg/L) were observed using transmission electron microscope, TEM. The electron density is evenly distributed in *Proteus* cells, which is a typical morphological characteristic in these normal cells. While in treated cells, there were a randomly distribution in nuclear area and obvious bright electron areas in the center of bacteria cells, in addition to there was cell wall rupture investigated (Figure 3). Concerning to *Klebsiella* sp. cells, it was also noticed that, a randomly cytoplamic materials distribution in addition to a rupture in cell wall of some treated *Proteus* cells and a leakage of cytoplamic materials (Figure 4).

3.1.3. Silver Nanoparticles Affect on the DNA of Bacterial Isolates

The amount of DNA from normal bacterial cells and AgNPs - treated bacterial cells was evaluated by agarose gel electrophoresis. Agar gel electrophoresis images as shown in (Figure 5) revealed that there are destructive effects of AgNPs on both pathogenic bacteria genome. The results showed that, there was single band for normal *Proteus* cell detected at a distance 1.5 Cm of about molecular weight 2500 bp, while DNA of AgNPs-treated *Proteus* cells was fragmented at three bands at a distances 4.9, 5.5 and 6.3 with molecular weights 950, 850 and 740 bp respectively. Concerning to *Klebsiella* sp., there was also one band of normal DNA cells at distance 1.5 cm and molecular weight 2500 bp, while in AgNPs-treated cells, DNA was detected at distances 4.8, 5.4 and 6.2 with molecular weight 950. 850 and 740 bp.

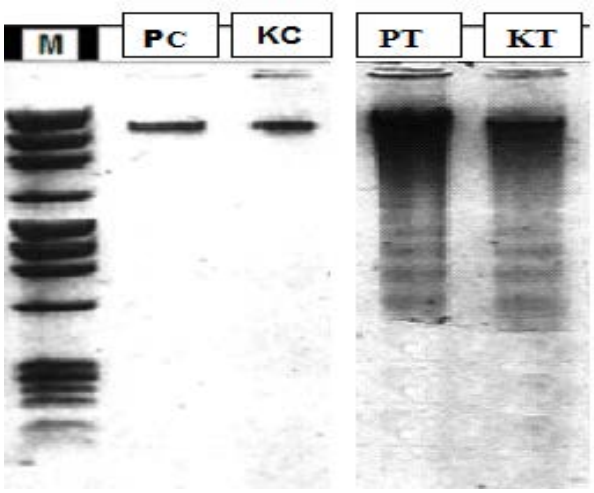

Figure 5. The amount of DNA from normal *proteus* cells (PC) and normal *Klebsiella* cells (KC) and bacterial cells treated by AgNPs (PT) and (KT) on agarose gel

4. Discussion

Emerging infectious diseases and the increase in incidence of drug resistance among pathogenic bacteria have made the search for new antimicrobials inevitable. In the current situation, one of the most promising and novel therapeutic agents are the nanoparticles. The unique physiochemical properties of the nanoparticles combined with the growth inhibitory capacity against microbes has led to the upsurge in the research on nanoparticles and their potential application as antimicrobials. From centuries metals such as silver have been used for treating burns and chronic wounds, and copper has been used to make water potable. It is quite evident that some of the metallic compounds possess antimicrobial property. Recently, the confluence of nanotechnology and biology has brought to manipulate the metals in the form of nanoparticles that could have a potential antimicrobial affects. *Proteus sp.* and *Klebsiella* sp. have been identified as important common pathogens for many human infections. Results showed that AgNPs exhibited antimicrobial activity with MIC 10 mg/L for *Klebsiella sp.* and *Proteus sp*. Some researchers found that 10 µg /ml concentration of AgNO3 and Ag nanoparticle was able to inhibit bacterial growth and create a zone of 0.8 cm and 1.7 cm respectively [13]. It was also demonstrated that in case of 12 hr treatment using Ag nanoparticles, 100 per cent growth inhibition of *E coli* recorded from 30ug/ml to 50 µg /ml, But AgNO3 showed lower performance, even in the 50 µg/ml concentration; more colonies grew on the plate [13]. While other study has shown that nanoparticles of Ag do not have significantly different antimicrobial activity towards Gram positive and Gram negative bacteria [14]. Concerning to other tested metals nanoparticles in this study no detection for the antibacterial activity of PtNPs, PdNPs or CuNPs against both bacterial isolates at all tested concentrations was recorded. This results agreed with [15] who demonstrated that the colloidal platinum nanoparticles CPtN up to 2.5 mM has no antibacterial activity. While other study indicate that undoped and palladium doped titania nanoparticles exhibited better antibacterial activities against *Escherichia coli*.

Morphological structure of normal *Proteus* and *Klebsiella* cells was compared with AgNPs-treated cells at a concentration (10 mg/L) using transmission electron microscope. Results showed that, there were randomly distribution in the nuclear area and obvious bright electron areas in the center of *Proteus* cells. It was also noticed that, a rupture in the cell wall of some treated cells. Also, there was damage of cell wall of some treated *Klebsiella* cells and leakage of the cytoplamic content was investigated. Other studies [16,17,18] reported that the surface of the cell walls of *E. coli* treated with silver nanoparticles were severely damaged compared to untreated *E. coli* Cell wall rupture due to silver ions and silver nanoparticles.

In this study the amount of DNA from normal bacteria cells and bacteria cells treated by AgNPs was evaluated by agarose gel electrophoresis in a trail to understand the action mechanism of the antibacterial activity of silver nanoparticles. Results revealed that there was a destructive effect of AgNPs on treated *Proteus* and *Kelbsiella* genome that were investigated as many fragments with different molecular weight on agarose gel. Pervious experimental evidence suggests that DNA loses its ability to replicate once the bacteria have been treated with Ag ions or Ag nanoparticles, the bactericidal activity of these nanoparticles depends on their stability in the culture medium, since this imparts greater retention time for interaction of bacterium and nanoparticles [19]. Although some literature reports bacterial cells with negative charge can interact with silver nanoparticles with positive charge due to electrostatic interaction, there are no complete reports about how silver nanoparticles affect the process of damaging bacteria and the impact to bacterial DNA [11]. Additionally, Silver nanoparticles lead to the formation of “pits” in the cell walls of the bacteria and can enter into the periplasm through the pits and destroy the cell membrane. Also, it was found that, silver nanoparticles not only condense DNA, but also combine and coagulate with the cytoplasm of damaged bacteria, which results in the leakage of the cytoplasmic component. Silver nanoparticles may cause the condensing of DNA, resulting in a loss of replication and degradation of DNA, thereby inhibiting bacterial growth. There is condensed DNA in the center of the bright electron areas which appears in the middle of bacteria cells [10]. Several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulfur-containing proteins, leading to the inhibition of enzyme functions [20]. In this study TEM of silver nanoparticles investigated its dimension size was ≈ 38nm that could related to the action mechanism on cytoplamic and DNA structures and this agreed with study that reported, the micromolar levels (in the size range of 10-15 nm) of Ag+ ions have been reported to uncouple respiratory electron transport from oxidative phosphorylation, inhibit respiratory chain enzymes, or interfere with the membrane permeability to protons and phosphate [21]. In addition, higher concentrations of Ag+ ions have been shown to interact with cytoplasmic components and nucleic acids [22,23].

5. Conclusion

The antibacterial activity of silver, platinum, palladium and copper nanoparticles on *Proteus sp.* and *Klebsiella* sp. were investigated. The morphological and DNA structures of the bacterial cells following treatment with potentially effective AgNPs were detected. The experimental results indicated that 10 mg/l AgNPs (size 38 nm) was MIC at which both bacterial cells were inhibited and the cellular components became disorganized and scattered from their original ordered and close arrangement in addition to cell wall rupture based on TEM observation. Also AgNPs caused a destructive effect on DNA, resulting in a loss of replication and degradation of DNA, thereby inhibiting

bacterial growth. No detection were investigated for other tested nanoparticles.

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