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Investigating the effect of biosynthesized silver nanoparticles as antibiofilm on bacterial clinical isolates

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ABSTRACT

Silver nanoparticles showed enhanced biofilm inhibitory activity of clinical pathogens. Eleven isolates (45.8%) of *E. coli* bacteria were obtained from 24 wound specimens. Silver nanoparticles biosynthesized by *E. coli* culture supernatant with exhibition dark brown color after 24 hr of incubation. Scanning electron microscopy showed that Ag-NPs spherical particles and its size were (14.2–67.8) nm and its average was 33.6 nm. X-ray diffraction shows one high peak at 2ø (32.5°) compared with standard data. Fourier transform infrared spectroscopy analysis of Ag-NPs exposed the strong band at 1367.53 corresponds to OH-bend which influences the synthesis and stability of Ag-NPs, whereas the stretch for Ag-NPs found at 518.58 cm^{-1}. The antibacterial effect of Ag-Nps against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus* showed the inhibition zone of 10, 11, 13, and 10 mm, respectively. Strong biofilm formed by isolates of *E. coli* exhibited as black colonies on Congo red agar, while pink colonies on it with Ag-Nps, indicating a loss of biofilm formation ability in all tested bacterial isolates. Antibiofilm of 10 mM Ag-Nps by Microtiter plate exhibited lower biofilm inhibition against *S. aureus* reached to 22.2%, while 36.2% against *E. coli*, 30.4% for *K. pneumonae*, and *P. aeruginosa* 94.7%. Analysis of biofilm components after exposure to Ag-NPs by FT-IR reveals a low level of proteins, polysaccharides, lipids and nucleic acids compared with controls, then glucose formation measured and exhibited reduction of absorbance (0.146 nm) after treatment with Ag-Nps compared with control (0.347 nm), while the percentage of protein decreased completely (0%) compared with control (0.25%). The combined effect of Ag-NPs with antibiotics enhanced the antibiofilm activity which tested under inverted microscope (40X).

1. Introduction

Biofilms are communities of microorganisms attached to a firm surface. These adherent cells regularly embedded within a self-produced matrix of extracellular polymeric Substance [1]. The major problem is that biofilms are very resistant to host defense mechanisms and antibiotic treatment, compared to their planktonic cells [2].

Many previous studies have demonstrated the relationship between microbes and metals, such as bioremediation, biomineralization, microbial corrosion, and leaching. In accession to this, several microorganisms have been explored for their potential to synthesize metallic nanoparticles. The methodologies were found to be reliable, efficient, and eco-friendly, and avoid the disadvantage of chemical and physical synthesis [3].

Ag-Nanoparticles have drained substantial interest among the promising nanoproducts because of their unique properties and increasing utilization of different applications in Nanomedicine [4].

The effects of silver nanoparticles (Ag-NPs) on bacterial cell are complicated. However, direct morphological observation by electromicroscope gives us structural change in the bacterial cell. It may give us useful information for understanding the antibacterial action of Ag-Nps [5].

The ability of AgNPs to block bacterial growth, and to prevent the glycocalyx formation, was helpful to prevent bacterial adhesion and following the biofilm formed on the medical devices [6].

The aim of this search concerned with analyzing the antibacterial and *anti*-biofilm activities of biosynthesized Ag-NPs against some bacterial pathogens. The effects of combining antibiotics with AgNPs against pathogenic bacteria were also determined.

2. Materials and methods

2.1. Isolation and identification of E. coli

E. coli bacteria isolated from wounds samples of patients in a teaching laboratory at Medical City in Baghdad-Iraq. These samples

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Fig. 1. Api 20E system results for *E. coli.*

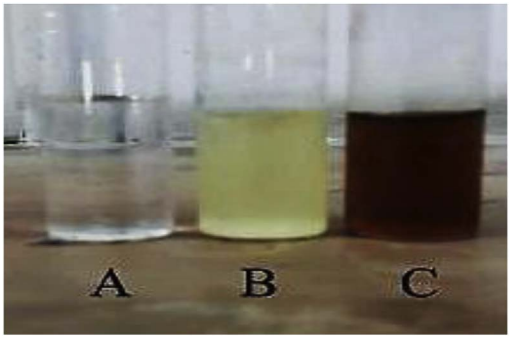

Fig. 2. Synthesis of Ag-Nps (a) silver nitrate (b) culture supernatant of *E. coli* (c) mixture of silver nitrate (10 mM) with bacterial supernatant and glucose.

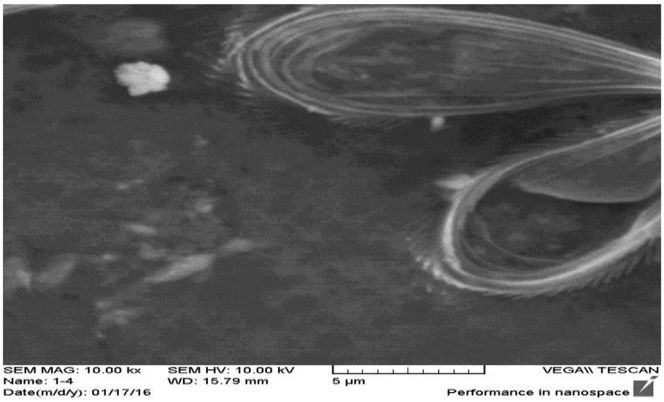

Fig. 3. SEM image of Ag- nanoparticles synthesized by *E. coli.*

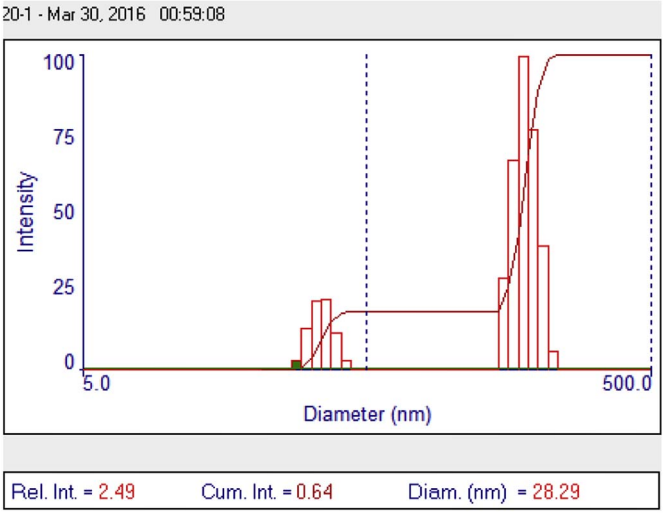

Fig. 4. Diameter of *Silver nanoparticles.*

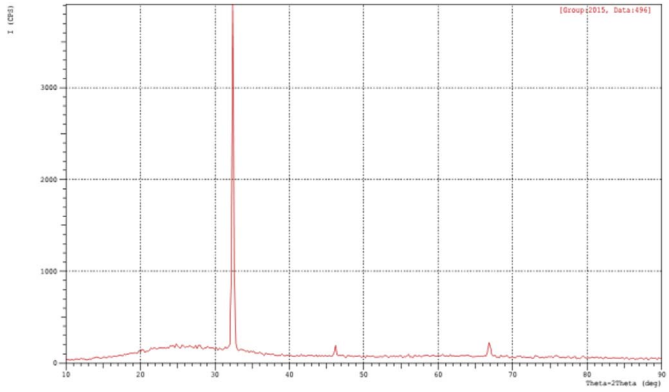

Fig. 5. XRD spectrum of synthesized silver nanoparticles.

isolated and identified on MacConkey, Nutrient and Blood agar media. After 24 h of incubation at 37 °C, morphological characterization (color, shape and size) recorded on the media and biochemical tests was tested according to [7].

2.2. Confirmation the identification of bacteria

A conventional API 20E system used to confirm the characterization of bacterial isolates.

2.3. Preparation of bacterial supernatant

The bacterial isolates cultured in nutrient broth, and incubated in a rotary shaker overnight at 37 °C. After 24 h, the culture centrifuged at 10,000 rpm for 10 min, then the supernatant collected for synthesis of silver nanoparticles [8].

2.4. Synthesis of silver nanoparticles by E. coli

Bacterial supernatant of ***E. coli*** with 10 mM concentration of $AgNO_3$ with 100 mM glucose mixed together in 1:1:1 proportion. The resulting solution was kept at 37 °C in a rotary shaker (at 200 rpm) [9].

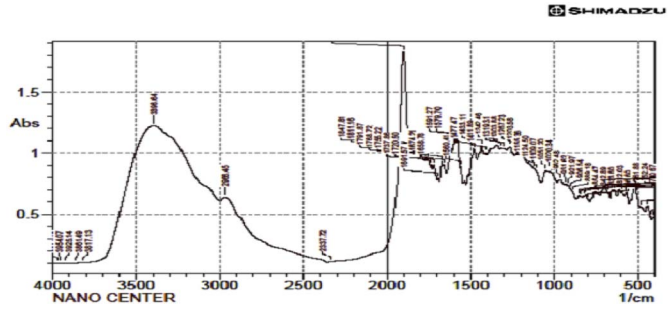

Fig. 6. Spectrum of FTIR of silver nanoparticles.