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SILVER NANOPARTICLES THAT SYNTHESIS BY USING *PSEUDOMONAS AERUGINOSA* SYNERGISTICALLY ACT WITH ANTIBIOTIC

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Abstract– Silver nanoparticles that synthesis by using bacteria have ability to act as antibacterial activity. *Local P. aeruginosa* isolates used to synthesis AgNPs. Antibacterial activity of AgNps were determined by minimum inhibition concentration it was 0.53 mg/mL used in well diffusion method on Muller Hinton. The results appeared that mean of inhibition for P. *aeruginosa* and *S. aureus* were 14.06, 14.22mm with AgNPs respectively. Antibiotic (Amox and Gent) were used with *S.aureus* these inhibit the growth of bacteria the inhibition zone of these antibiotic were increased when mixed with AgNPs, *P. aeruginosa* resist these antibiotic so using another (Mep) also the inhibition zone increased when mixed with AgNPs. The conclusion of this study the AgNPs act synergistically with antibiotics

INTRODUCTION

Antibiotic-resistant infections are already widespread in the word, the most common o gram-positive pathogens, a global pandemic of resistant *S. Aureus* and *Enterococcus* species currently poses the biggest threat. Vancomyci-resistant enterococci (VRE) and a growing number of additional pathogens are developing resis-tance to many common antibiotics. The global spread of drugresistance among common respirator y pathogens, including *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, is epidemic. Gram-negative pathogens are particularly worrisome because they are becoming resistant to nearly all the anti- biotic drug options available, creating situations reminiscent of the pre-antibiotic era (Michael *et al*., 2014). The emergence of MDR (and increasingly pan-resistant) gram-negative has affectedpractice in every field of medicine. The most serious gram- negative infections occur in health care settings and are most commonly caused by Enterobacteriaceae, *Pseudomonas aeruginosa*, and *Acinetobacter* MDR gram-negative pathogens are also becoming increasingly prevalent in the community. These include extended-spectrum beta-lactamase-producing *Escherichia coli* and *Neisseria gonorrhoeae* (Rossolini *et al*., 2014) so the need for alternative material than drugs is appeared Green Synthesis Using Bacteria as a medium. Bacteria are known to produce inorganic materials either intracellular or extracellularly.This makes them potential biofactories for the synthesis of nanoparticles like silver and gold. Silver is wellknown for its biocidal properties, some bacteria are known to be silver resistant and can accumulate silver on the cell wall to as much as 25% of their dry weight biomass, thus suggesting their use in industrial recovery the use of prokaryotic bacteria as nanofactories was first studied. First noble metal nanoparticle synthesis, using bacteria, was done using silver resistant bacterial strains *Pseudomonas stutzeri* AG259, which were cultured in high concentrations of silver nitrat (Slawson *et al*., 1992; 2016) silver nanoparticles have become of major interest for their antibacterial properties and are already integrated into applications such as wound treatment, sterilization, food sanitation, antibacterial

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textiles, and more recently drug delivery. In fact, silver nanoparticles exhibit a broad spectrum of antibactericidal and antifungicidal activities making them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food, and textiles, (Rauwel *et al*., 2015) the aim of this study to know the effect of nanoparticles with drug on growth of bacteria

MATERIALS AND METHODS

Bacterial isolate

Pseudomonas aeruginosa and *Staphylococcus aureus* isolates were taken from advance lab of microbiology, these isolates were diagnosed by biochemical tests according and confirmed by vitek 2 system (Biomerieux) (MacFaddin, 2000).

Media

Muller-Hinton (MHA) agar medium was ready for conferring to the manufacturing company and it was used in antimicrobial susceptibility testing. Nutrient Agar Medium, it was used for cultivation of the bacterial isolates when it was necessary. Nutrient Broth, This medium was used to grow and preserve the bacterial isolates. Brain Heart Infusion medium was prepared according to the manufacturing Instructions (Himedia, India) (MacFaddin, 2000).

Biosynthesis of Silver Nanoparticles b*y Pseudomonas aeruginosa*

Pseudomonas aeruginosa was initially grown at 37 ^{0}C for 24 hr in a 1000 mL Erlenmeyer flask that contained nutrient broth in a shaker incubator set at 200 rpm. Following bacterial growth, all the culture suspensions were incubated with aqueous 5 mM solutions of $AgNO_3$ at 37 ^{0}C in a shaker incubator at 200 rpm in the dark, the reactions was carried out for up to 120 hr for 5 days. The synthesis of AgNPs was detected by visual inspection of the culture flask for a change in the color of culture medium from yellow to brown/green. Extracellular AgNPs were separated from bacterial cells by centrifuging aliquots of culture supernatants at 3000 rpm for 6 min at 25 ^{o}C. The AgNPs suspensions were diluted 10 times using deionized water at every time point and UV-vis spectra were obtained, the samples were prepared by precipitating AgNPs obtained after 20 hr of biosynthesis at 13,000 rpm for 20 min, followed by four washings with deionized water and drop casting the samples onto a glass substrate (Song and Kim, 2009).

Antibacterial activity of silver nanoparticles

A volume 0.1 mL of the standard inoculums 1.5 X 10^8 cell / mL of the test bacterial (10 isolates for each *S.aureus* and *P.aeruginosa*) isolated was streak on Mueller Hinton Agar with a sterile swab and permitted to dry. Then, by using 6mm. diameter wells were bored using cork borer in the MHA AgNPs (2.12, 1.06, 0.53, 0.265, 0.1325) were introduced into each well and allowed to stand for 1 hr, at room temperature to diffuse the AgNPs into medium before incubation at 37^{0}C for 24 hr. The Inhibition zone diameter (ISD) was measured by obvious ruler to nearest mm (Okoli and Iroegbu, 2004). Antimicrobial susceptibility test by agar disk method according (Clinical and Laboratory Standards Institute, 2017).

Drug mix with AgNps

Three drugs(Acamoxil 250 mg Aki company, and Getmacin Sandoz company) were dissolved with sterile distill water ,and then added with 0.53 mg/ mL from AgNPs by using by agar disk method and used for the *S. aureus* isolates while (Mepenex Acino) with *P. aeruginosa*.

RESULTS AND DISCUSSION

Silver nanoparticles synthesis by using *Pseudomonas aeruginosa* as prepared by (Alsarhan, 2017) the categories of this nanoparticles was detect as previous study (the size of particles 32.7 nm). Antibacterial activity of silver nanoparticles was study on *S. aureus* and *P. aeruginosa* isolates. The concentration 0.53 mg/mL was determined as minimal inhibition concentration for bacteria this agree with (Abd, 2018; Raheem *et al*., 2018). The compulsory of the particles to the bacteria depends on the interaction of the surface area available. With a smaller particle size, a large surface area will have a bactericidal influence. Secondly, Ag NPs are capable to enter the bacteria by maybe interacting with sulfur- and phosphorus-containing composites such as DNA and cause additional damage (Matsumura *et al*., 2003). Thirdly, the AgNPs discharge silver ions, which contribute to the bactericidal effect (Kim *et al*., 2007). The device of inhibition by silver ions on microorganisms is partlyacknowledged. It is believed that DNA loses its replication capability and cellular proteins

convert in activated upon silver ion treatment (Gupta *et al*., 2008). In addition, higher concentrations of Ag+ ions have been shown to interact with cytoplasmic constituents and nucleic acids (Kim *et al*. 2007).

Table 1. Antibacterial activity of silver nanoparticles against *Pseudomonas aeruginosa and Staphylococcus aureus*

Bacteria	Mean of inhibition zones by mmM±sd
Pseudomonas aeruginosa	14.06±3.5
Staphylococcus aureus	14.22±3.4

Significant p≤0.05

Table 2 appeared that silver nanoparticles increase zones of inhibition when mixed with antibiotic this may be due to their sub-microscopic dimensions less than 1 um and the unique properties which emerge at these nanostructures easily interface with biological molecules, as nucleic acids and proteins. Among the nanostructures which are increasingly being used as biological sensors and delivery vehicles for therapeutic agents, nanoparticles are of particular interest. Beneficial characteristics of metallic nanoparticles include size and shape

Table 2. Mean of inhibition zones of antibiotic alone and with silver nanoparticles against *Staphylococcus aureus*

Antibacterial agent	Mean of inhibition zones by mmM±sd
Amoxcillin	18.61±2.3*
Amox +silver	21.833±3.8*
Gent	19.16±4.7*
Gent+silver	21.72±5.1*

Significant differences among groups at p≤0.05

P. aeruginosa isolates. Were appeared resist to antibiotic (Amox and Gent) so using another antibiotic meropenem according (Clinical and Laboratory Standards Institute 2017). The isolates were intermediate sensitive the diameter of inhibition (16.3 mm) to mepenex but when mix with seliver become sensitive (30.06mm) the conclusion of this study the silver nanoparticles act synergistically with antibiotics.

Table 3. Mean of inhibition zones of mepenex alone and with silver nanoparticles against *Pseudomonas aeruginosa isolates*

Antibacterial agent	Mean of inhibition zones by mmM±sd
Mepenex	16.31±3.9*
Mepenex+silver	30.06±2.3*

Significant p≤0.05

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