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THE EFFECTS OF ENZYME NANOPARTICLES ON ADHESION OF PATHOGENIC BACTERIA

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Abstract

One isolate of *Pseudomonas aeruginosa* was isolated from 10 isolates of clinical samples, the isolated showed different sensitivity against antibiotics, showed its ability to produce Biofilm and the inhibitory activity of the lipase enzyme was studied in the growth of *Staphylococcus aureus* and *Escherichia coli*, where the results showed no inhibitory effect on the growth of bacteria. The inhibitory effect of nanoparticles was studied in the growth of *S. aureus* and *E. coli* and recorded the highest inhibitory diameter of 18 millimeters Microgram / ml and when mixed lipase and silver nanoparticle against bacterial growth the highest inhibitory in concentration 100% was 25 mm.
***Keywords*:** *Pseudomonas aeruginosa,* MDR, Biofilm formation, Nanoparticles.

Introduction

The *Pseudomonas auer*oginosa is an important bacterial species because it is widespread in nature and causes many diseases for humans and animals including bacteremia, Urinary Tract Infection, Endocarditis, Wound and Burn infection (Bhasin *et al.,* 2015), It is an opportunistic nurse and rarely causes the disease in healthy people, but it is a real danger to patients who are in hospital, especially with patients who suffer from immune deficiency, such as people with AIDS, as well as those with burns. The most important bacterial species that cause known infections in hospitals Nosocomial infection (Mulcahy *et al.,* 2011).

These bacteria have high resistance to various antibiotics, making them difficult to treat, as well as having a number of agents, including exotoxin A, which is responsible for tissue necrosis and exoenzyme S, with the other adhesion factors responsible for stability of bacterial adhesion to host cells, Las B elastase, Alkalin protease, protease is responsible for tissue breakdown, Pyocyanin, Phospholipase, Rhamolipid, and Biofilmes.

Biomembrane is one of the virulence factors of pathogenic bacteria. It is a group of bacterial cells that are covered with a layer called polysaccharide, which helps bacteria adhere to the lung in patients with vesicular fibrosis and can also be attached to surfaces. Biomembrane represents a resistance shown by bacteria against antibiotics or against physical therapy (Wolska and Szweda, 2009), Biomembrane provides protection against harsh environmental conditions such as drought and various antibiotics (Holban *et al*., 2013). Many bacterial species are present within a single membrane layer by improving their living conditions in this layer by competing for location and food (Mercedes and Ricardo, 2016), the aim of this study:

1- Effects of Silver Nanoparticles on Biofilm Formation

2- Antibacterial Effects of Silver Nanoparticles

Materials and Methods

Isolation and diagnosis of bacteria

The 10 isolates of Pseudomonas bacteria were collected from various pathologies including (middle ear infection, wounds, burns) for the period from November 2018 till December 2018 several specialized laboratories in Baghdad. The isolates were diagnosed using of biochemical tests and Vitak 2 System for final diagnosis (Stewart and Costerton, 2001).

Antibiotic sensitivity test

Antimicrobial susceptibility testing with antibiotics

From the bacterial exponential growth (~16 hours), a cell suspension in saline was adjusted to 0.5 McFarland and inoculated in Muller Hinton Agar–MHA (Sigma-Aldrich) using agar diffusion method. After 15 minutes of being allowed to stand, discs recommended by the Clinical Laboratory Standards Institute (CLSI) were placed on the plate and incubated at 35°C (±2) for 16–18 hours. The antibiotics tested were: Erythromycin, Ciprofloxacin, Chloramphenicol, Doxycycline, Amikacin, Tobramycin, Azthromycin, Clindamycin. Bacterial susceptibility to these antibiotics were verified by measuring the diameter of the inhibition zones formed and then interpreted according to values set by the CLSI.

Kirby baure (Flemming and Wingender, 2010).

Synthesis of Silver nanoparticles

Silver nitrate is prepared according to (DeLeon, 2014; Shobha, 2015).

Investigation of Biofilm-produced isolates

The *P. aeruginosa* bacteria were grown in the media of the Trypton Soy Broth soybean for 24 hours in test tubes and in 5 ml of tube and added 1 ml of Lipase-enzyme concentration (40% microgram/ mL), silver nanopartical (60% microgram / ml) and a combination of lipase and nano-silver nitrate (100% µg / mL) for each tube. The positive control tube contains the bacterial fertilized medium. The negative control tube contains only media. After the incubation period, the medium was washed out and the tubes were washed with pH 7.3 phosphate solution and then dyed with Crystal Violet 1% for 5 minutes. The dye was removed and the tubes were washed away from the rest of the dye and the tubes were left inverted position to dry (Deka, 2014). The results were recorded as follows:

The result (-) was given if there was no production of the biomass (negative control).

The result (+) was given if the composition of the biomass was weak.

The result (++) was given if the composition of the biomembrane was average

Study of the effect of Lipase-enzyme and silver nanopartical and their combination against *E. coli* and *S. aureus*

To determine the effectiveness of Lipase -enzyme, the silver nanoparticle and their mixture to the isolated bacteria, the Agar well diffusion method was used. The bacteria *S. aureus* and *E. coli* were caught, and 0.1 mL of it was taken to the center of Mueller Hinton agar, In addition to each hole, 75 microliters of Lipase were added to each hole. The silver nanoparticles and mixed with concentrations of 20, 40, 60, 80, 100 µg / mL, and 75 µl of distilled water was added to the control hole.and incubated at a temperature of 37 m for 24 h. The bacterial growth inhibitor was measured around each hole (Balouiri,2016).

Results and Discussion

After testing for the diagnosis of bacterial isolates, it was ascertained that 1 isolate belonging to *P. aeruginosa* were isolated from different sources.

Antibiotic sensitivity test of *P. aeruginosa*

The bacteria showed a resistance to antimicrobial Erythromycin (100%), while the results showed sensitivity of the bacteria to the antimicrobial Ciprofloxacin with an inhibition diameter of 30.50Millimeter followed by Chloramphenicol with 26.26 mm inhibition, then Doxycycline at 25 mm inhibition, Tobramycin and Amikacin at 19 mm and 18 mm inhibition respectively, and Azthromycin and Clindamycin with an inhibition rate of 9,16 mm respectively (Jeevan *et al.*, 2012; Rahim and Mohamed, 2015) as shown in Figure (2).

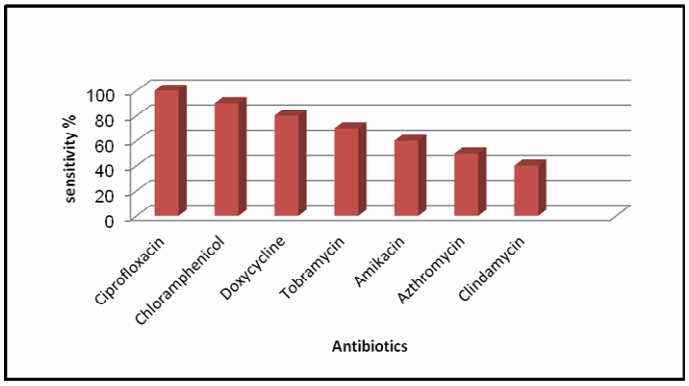

Fig. 1 : Percentage of resistance to *P. aeruginosa* isolates of antibiotics

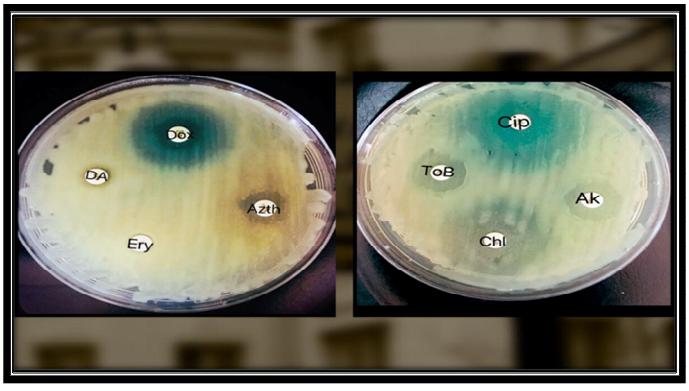

Fig. 2 : Antibiotic sensitivity test

Quantitative investigation of biofilm inhibition by test tube method

The *P. aeruginosa* was grown in the media of the Trypton Soy soybean after the medium was removed and the tubes were dyed with a UV dye of 0.1%. The amount of pigment in which the tubes were stained varied. The positive control tube produced a dense biomass (+++), The negative control tube did not have a biofilm (-), treated tube with lipase concentration of 40%), a medium-dynamic membrane (++), a silver nano tubes treated with concentration (60%), In a combination of 100% lipase enzyme and 100% silver nanoparticles, there was no biofilm appeared (Konaté *et al.,* 2012) (Figure 3).

Fig. 3 : Quantitative investigation of biofilm inhibition by test tube method

Study of the Effect of Silver Nanoparticle and Lipase on *S. aureus* and *E. coli*

The results of the study of the effect of nanoparticles in agar well diffusion method showed that the concentration of (100, 80, 60, 40, 20%) µg / ml had a inhibitory effect of *S.aureus* bacteria and the diameter of the 16 mm inhibitor was 100% microgram / Ml, while *Ecoli* had no inhibitory effect at these concentrations used (Fig. 4),and no appear inhibitory effect of lipase enzyme on *S. aureus* and *E. coli.* Antibacterial susceptibility to the ability of nanoparticles to behave as a bacterial antimicrobial agent has been attributed to the elimination of bacteria by combining it with the bacterial cell membrane and then penetrating the bacterial cell. The association between nanoparticles and cell membrane is attributed to the presence of proteins in the membrane. Which is a container on the sulfur, which leads to the destruction of the cell membrane and then enter the nanoparticles into the cell and union with the structures containing the groups of phosphorus and sulfur, such as respiratory enzymes and DNA, leading to the death of the bacterial cell (Konop *et al.,* 2016; Nowroozi *et al.*, 2012).

The mixture showed a synergistic effect against the growth of bacteria. The diameter of the largest inhibition was 22 mm while the diameter of the smallest inhibition was 14.1 mm (Fig. 6).

The reason why binary materials are more effective than if they are used individually is that one of these substances may contain one or several chemical compounds that increase the inhibitory effectiveness of an active compound in the other material. Therefore, when thinking about making a mixture of materials, it is preferable to study the compounds of each material individually or mono and then mix those that have a synergistic effect in order to reach the desired result of this process Such a case is known in pharmacology in antibiotic studies. When blending with bleomycin and camptithecin, which have anti-DNA effect

when used individually, there is a decrease in this activity or a lack of antimicrobial use. Some compounds are more effective when mixed, while others are less effective. Effectiveness or disappearing (Pezzuto *et al.,* 1991).

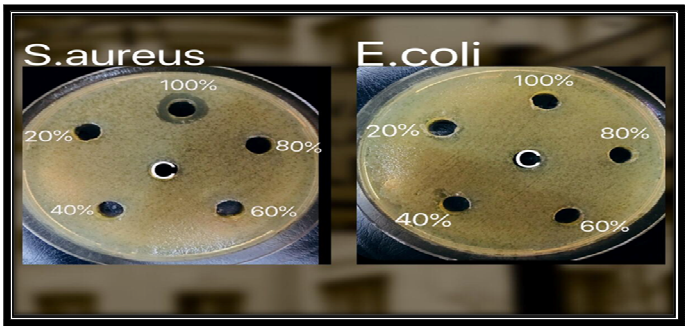

Fig. 4 : Shows the effect of silver nitrate against the pathogenic bacteria *E. coli and S. aureus*

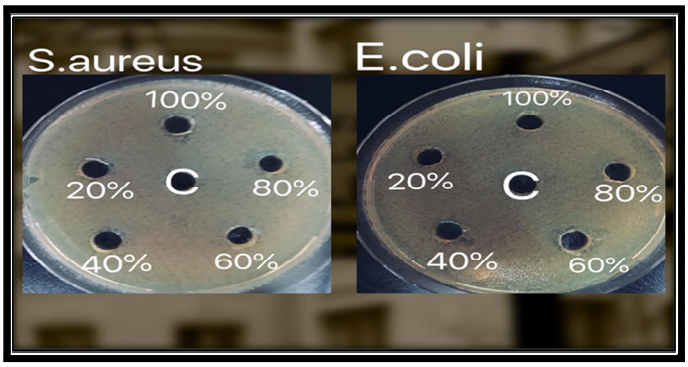

Fig. 5 : Shows the effect of Lipase-enzyme against the pathogenic bacteria *E.coli* and *S.aureus*

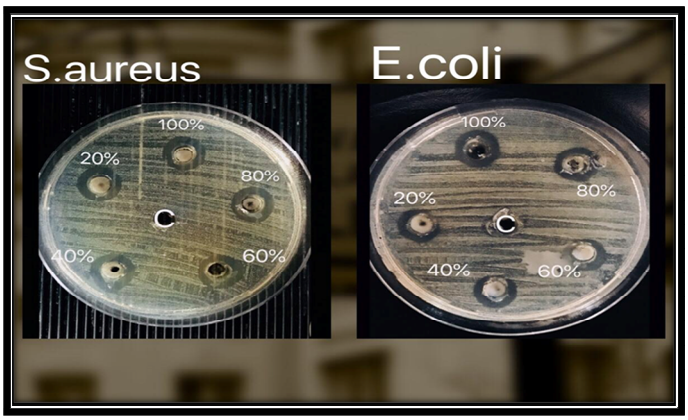

Fig. 6 : Shows the effect of the silver nanopartical mix with the lipase enzyme against the *E. coli* bacteria and *S. aureus*

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