The synergistic effect of biosynthesized gold nanoparticles with antibiotic against clinical isolates

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Abstract

Background: Antibiotic resistance is a life threating problem and the need for an alternative is increasing worldwide.

Objective of this study is: Detecting the combination effect of AuNps with Amoxicillin/clavulanate (AMC) antibiotic against antibiotic resistant clinical bacterial isolates.

Materials and methods: Gold nanoparticles were biosynthesized using food origin *Citrobacter freundii* isolate (C2) which was isolated from chicken meat samples by pour plate method and identified using cultural characteristics, biochemical tests and the identification to the species level was completed by Vitek-2 system and this identification was confirmed by sequencing of the 16 s r RNA. The biosynthesis of gold nanoparticles was optimized in order to achieve the finest AuNps with a diameter range (30-60) nm. and the biosynthesized gold nanoparticles were characterized by visual observation and then characterized using various characterization techniques: Uv-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR) analysis, atomic force microscopy (AFM) analysis and scanning Electron Microscope (SEM). The combination effect of AuNps with AMC antibiotic was detected against clinical bacterial isolates.

Results: The results revealed the biosynthesized AuNPs were roughly spherical and poly-dispersed, and they were highly effective with concentration (62.5 µg/ml) that inhibit the bacterial growth, MIC values of AMC antibiotic against clinical isolates were determined as 500 µg/ml, while the combination of gold nanoparticles and AMC had wide spectrum of antibacterial activity against different isolates of the bacteria that used in this study.

Conclusion: There was a significant synergistic effect between the biosynthesized gold nanoparticles when used in combination with antibiotic where the minimum inhibitory concentrations of the combination (AuNps/AMC) were less than its concentration when each of them (AuNps) or (AMC) used separately.

Key words: Gold nanoparticles, *Citrobacter*, Synergistic effect, Biosynthesis.

Introduction

A new science that mostly involves the production, processing and application of structures, devices, and systems under adjusting shape and diameter at the nanometre range (1) . The nanotechnology is a science that deals with designing Nano-material by modulation of the particles at atomic level with the cost that does not exceed the cost of the raw material (2). Nanoparticles cover wide-ranging area from uses (3). One of best common usage of nanotechnology is Nano-medicine, which used in the field of health care. Nano-medicine makes usage of nanomaterial's, and Nano electronic biosensors. The benefits of nanotechnology application in medical field have many potentially valuable for all mankind (4).

Certain Nano-powders own antimicrobial properties. When these powders contact cells of *E. coli*, or other bacteria species and viruses, over 90% are eradicated within a few minutes. Because of their antimicrobial activity, nanoparticle of titanium dioxide and silver are used as coatings for surgical masks. Zinc nanoparticles can improve the activity of ciprofloxacin against bacteria, and decrease the antibiotic resistance by interfering with different proteins that are relating to the antibiotic resistance (5).

To improve the capability of nanoparticles against bacterial is to combination them with antibiotics. For instance, Nanoparticles mixed with vancomycin or with aminoglycoside show better antibacterial activities against resistant strains as compared to antibiotics and nanoparticles alone (6). However, another strategy is to use Nanoparticles in combination with existing antibiotics to fight multi drug resistant bacterial infections (7). The aim of this study was to assume that nanoparticles show synergistic effect with antibiotics in combating multi drug resistant bacteria.

Materials and Methods

Food origin *Citrobacter freundii* isolate (C2) was isolated from chicken meat samples by pour plate method and identified using cultural characteristics, biochemical tests and the identification to the species level was completed by Vitek-2 system and this identification was confirmed by sequencing of the 16 s r RNA in a previous study (8). Gold nanoparticles were biosynthesized using *C. freundii* isolate (C2) in an optimized condition in order to achieve the finest AuNps with a diameter range (30-60) nm. and the

biosynthesized gold nanoparticles were characterized by visual observation and then characterized using various characterization techniques: Uv-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR) analysis, atomic force microscopy (AFM) analysis and scanning Electron Microscope (SEM) previously to this research (9).

Minimum Inhibitory Concentration (MIC) Determination

The antimicrobial efficacy of AuNPs, Amoxicillin/clavulanic acid and Amoxicillin /clavulanic acid + gold nanoparticles were examined using the standard broth dilution method. The MIC was determined in nutrient broth (NB) using serial two-fold dilutions in concentrations ranging from 15 µg/ml to 500µg/ml with adjusted bacterial concentration (1×10^8 CFU/ ml, 0.5 McFarland's standard). The positive control used in this study contained NB medium with tested bacterial concentrations while negative control contained only inoculated broth and the time and temperature of incubation being 24hrs and 37°C respectively. The MIC was the lowest concentration of antimicrobial agents that visually inhibits 99% growth of microorganisms. The MIC was noted by the visual turbidity of the tubes both before and after incubation (10).

Results and Discussion

MIC of AuNPs

The MIC of AuNPs was determined against nine clinical isolates of *Escherichia coli* (E1, E2 and E3), *Pseudommonas aeruginosa* (P1, P2 and P3) and *Staphylococcus aureus* (S1,S2 and S3) which were 62.5 µg/ml for (E1, E2, E3, P1, P3, S1 and S2) and 31.25µg/ml for (P2 and S3). According to the result mentioned it was concluded that the gold nanoparticles were highly effective with concentration (62.5 µg/ml) that inhibit growth of the nine isolates of the three bacterial species.

Evaluation of Antibacterial Activity of Amoxicillin/ clavulanate

The antibacterial activity of Amoxicillin/ clavulanate against 9 isolates of *E. coli, P. aeruginosa* and *S. aureus* was determined using standard broth dilution method**.** MIC values of AMC against clinical isolates were determined as 500µg/ml for *E. coli* (E1 and E2) and *S. aureus* (S1, S2 and S3) while it was 1000µg/ml for *P. aeruginosa* (P1, P2 and P3) and *E. coli* (E3).

Evaluation of Antibacterial Activity of Gold nanoparticle

Standard broth dilution method was used to detect the antibacterial activity of AuNPs, where serial dilutions of AuNPs (500, 250, 125, 62.5, 31.25 and 15.625) µg/ml were prepared and the antibacterial activity was determined depending on minimum inhibitory concentration (MIC), and Table (1) shown these results.

Table (1): Antibacterial activity of AuNPs against clinical isolates of *E. coli, P. aeruginosa* and *S. aureus*

Bacterial Isolates	Gold nanoparticles concentrations					
	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	31.25µg/ml	15.625µg/ml
E1	+	+	+	+	-	-
E2	+	+	+	+	-	-
E3	+	+	+	+	-	-
P1	+	+	+	+	-	-
P2	+	+	+	+	+	-
P3	+	+	+	+	-	-
S1	+	+	+	+	-	-
S2	+	+	+	+	-	-
S3	+	+	+	+	+	-

*The positive (+): means inhibition of bacterial growth

*The negative (-): means bacterial growth

The MIC of AuNPs was determined against nine clinical isolates of E. *coli* (E1, E2 and E3), *P. aeruginosa* (P1, P2 and P3) and *S. aureus* (S1,S2 and S3) Which were 62.5 µg/ml for (E1, E2, E3, P1, P3, S1 and S2) and 31.25µg/ml for (P2 and S3). According to the result mentioned it was concluded that the high effective of gold nanoparticles with concentration (62.5 µg/ml) that inhibit growth of the nine isolates of the three bacterial species. Previous research about the biogenic AuNPs detected also the same pattern of antibacterial activity of gold nanoparticles against *E.coli* and *S. aureus* as noted the MIC values were 75 µg/ml for *E. coli* and 50 µg/ml (11). Another study reported by (12) who found the inhibition of *E.coli* and *S. aureus* by gold and gold nanoparticles. The mechanisms of gold nanoparticles effected on bacteria described

by many studies, as it may be due to interaction with the cell wall of bacteria that lead to the formation of pores in these walls, accumulation of the nanoparticles in the pits caused the permeability of the cell membrane (13,14). Other reason caused the death of the bacterial cells may be the effect of nanoparticles on the proteins in the cytoplasm of the cells which lead to disrupt the regulation in cell function , also the nanoparticles can effect on DNA replication which will disrupt the replication mechanism (15). The other suggestion of the mode of action of gold nanoparticles recorded in previous studies, that gold nanoparticle exerted the antibacterial effect in two steps: first, they changed the membrane potential and reduced adenosine triphosphate synthase (ATP) activities, thus reducing the metabolism process; secondly, they declined the subunit of the ribosome for tRNA binding, thus collapsing its biological mechanism. Small size of gold nanoparticles with enhanced surface area produced some electronic effects that are attracted to the bacterial surface and resulting directly interaction with the microorganism (16,17).

Evaluation of Antibacterial Activity of Amoxicillin/ clavulanate

The antibacterial activity of Amoxicillin/ clavulanate against 9 isolates of *E. coli, P. aeruginosa* and *S. aureus* was determined using standard broth dilution method**.** MIC values of AMC against clinical isolates were determined as 500µg/ml for *E. coli* (E1 and E2) and *S. aureus* (S1, S2 and S3) while it was 1000µg/ml for *P. aeruginosa* (P1, P2 and P3) and *E. coli* (E3) which were clarified in the Table (2).

Table (2): Antibacterial activity of Amoxicillin/ clavulanate against clinical isolates of *E. coli, P. aeruginosa* and *S. aureus*

Bacterial isolates	Amoxicillin/ clavulanate						
	1000µg/ml	500µg/ml	250µg/ml	125µg/ml	62.5µg/ml	31.25µg/ml	15.625µg/ml
E1	+	+	-	-	-	-	-
E2	+	+	-	-	-	-	-
E3	+	-	-	-	-	-	-
P1	+	-	-	-	-	-	-
P2	+	-	-	-	-	-	-
P3	+	-	-	-	-	-	-
S1	+	+	-	-	-	-	-
S2	+	+	-	-	-	-	-
S3	+	+	-	-	-	-	-

*The positive (+): means inhibition of bacterial growth

*The negative (-): means bacterial growth

Similar results were obtained by the study of (18), that the isolates of *P. aeruginosa* were high resistant to amoxicillin/clavulanate . *E. coli and S. aureus* isolates showed high resistance to Amoxicillin/ clavulanate in the recent studies (19, 20). This increasing in resistance levels may be due to the use of antibiotics in different sectors, such as human community, hospitals, farms and domestic animals, in human medicine, the misuse and overuse of antimicrobial agents can represent the main cause of increasing antibiotic resistance (20, 21).

The combination effect of gold nanoparticles with Amoxicillin/ clavulanate against clinical bacterial isolates

Comparison among the three treatments that used in this study (Amoxicillin/ clavulanate, gold nanoparticles, and combination Amoxicillin/ clavulanate + gold nanoparticles) was depended to determine the best one that showed antibacterial activity using standard broth dilution method. The data of testing antibacterial activity against *E. coli, P. aeruginosa* and *S. aureus* revealed that the combination of (Amoxicillin/clavulanate + gold nanoparticles) showed the highest antibacterial activity among the three treatments tested in most isolates, sub inhibitory concentrations of AuNPs were mixed with all inhibitory concentrations of Amoxicillin/clavulanate and the result revealed that all of them were effective as antibacterial agent of 100% of isolates that used in this study. The synergistic effect appeared when the combination depended. This data is shown in Table (3).

Table (3): The synergistic effect of AuNPs with AMC antibiotic against clinical isolates of *E. coli*, *P. aeruginosa* and *S. aureus*

Bacterial isolates	Amoxicillin/clavulanate concentration						Concentration AuNPs
	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml	
E1	+	+	+	+	+	+	31.25µg/ml
E2	+	+	+	+	+	+	31.25µg/ml
E3	+	+	+	+	+	+	31.25µg/ml
P1	+	+	+	+	+	+	31.25µg/ml
P2	+	+	+	+	+	+	15.625µg/ml
P3	+	+	+	+	+	+	31.25µg/ml
S1	+	+	+	+	+	+	31.25µg/ml
S2	+	+	+	+	+	+	31.25µg/ml
S3	+	+	+	+	+	+	15.625µg/ml

*The positive (+) means inhibition of bacterial growth

The obtained data had shown that the combination of gold nanoparticles and Amoxicillin/clavulanate had wide spectrum of antibacterial activity against different isolates of the bacteria that used in this study. The higher activity was observed against target bacteria in the combination of recent study due to the gold nanoparticles enhanced the activity of Amoxicillin/clavulanate by conjugation with each other. Polymeric nanoparticles are highly attractive as drug delivery vehicles due to their high structural integrity, stability during storage, ease of preparation and functionalization, and controlled release capability (22). The combination technology was proved to be more effective the using separated antimicrobial agents in a previous study (23). There are several studies proved that the antimicrobial activity was increased when using combination of gold nanoparticles with antibiotics, antibodies, and probiotic rather than used nanoparticles alone or antibiotic alone (24, 25). The combination of gold nanoparticles with vancomycin showed potent inhibitor growth of *E.coli* (26). The antimicrobial activity of vancomycin, amoxicillin and penicillin G increased when used in combination with nanoparticles (27, 28).

Conclusion

There was a significant synergistic effect between the biosynthesized gold nanoparticles when used in combination with Amoxy\clavulanate antibiotic where the minimum inhibitory concentrations of the combination (AuNps/AMC) was less than its concentration when each of them (AuNps) or (AMC) used separately, and this combination may be used as an alternative drug to minimize the antibiotic concentration which needed for microbial eradication.

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