Antimicrobial activity of silver nanoparticles biosynthesized by *Streptomyces* spp.

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Abstract

Nanobiotechnology is a new emerging discipline of nanoscience created by pairing of biotechnology, and nanotechnology. Silver nitrate ($AgNO_3$) was used as precursor for biosynthesis of silver nanoparticles by *Streptomyces* spp.Silver nitrate was added with concentration 5 mMto biomass of *Streptomyces spp. (*MU-43 &SA-65)which distributed in sterilized flasks containingISP4 broth medium.This step was carried out under dark condition to avoid oxidation of AgNO3.Antibacterial activity of AgNPs were biosynthesized by each of the two isolates of *Streptomyces*spp(MU-43and SA-65) were examined for their antimicrobial activity against different types of pathogenic bacteria isolated,including both Gram positive and Gram negative bacteria were determined by disc diffusion method Antibacterial activity against tested bacteria was observed to be different regarding the source of biogenic AgNPs applied. The results showed that gram negative bacteria were higher sensitive than gram positive bacteria to AgNPs. Furthermore AgNPs fabricated by *Streptomyc*es MU-43 isolate had higher activity than AgNPs fabricated by *Streptomyces* SA-65 isolate. Synergism among antibiotics-silver nanoparticles biosynthesized by both *Streptomyces* strains (MU-43 & SA-65) showed that this synergism was efficient to inhibit the tested bacteria.

Keywords: Silver nanoparticles, Antimicrobial activity, *Streptomyces*, Biosynthesis.

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Introduction

Nanobiotechnology is a new emerging discipline of nanoscience created by pairing of biotechnology, and nanotechnology it's concerned with tremendous scope in development of pioneer materials in health, medicine, environment, economic, science and technology, and other sectors thus creating a largeshifts in the development of technologies. From which molecular-nanobiotechnology emerged to concern with the study of cellular mechanisms involved in nanobiosynthesis at molecular level and creation of complex molecular machines having the job to function certain tasks [**1**]. *Streptomyces* are the biggest source for antibiotics production, they constitute about more than 75% in antibiotic productivity, and these antibiotics have a significant application in the commercial and therapeutic fields [**2**, **3**]. Silver nanoparticles (AgNPs) have established important attention because of their different applications as antibacterial, antifungal agents, antioxidant and inhabit biofilm formation in biotechnology and bioengineering. Also they have application in catalysis, optics, electronics and other areas due to their unique size dependent optical, electrical and magnetic features. The synthesis of silver nanoparticles is widely studied by using chemical,physical and biological approaches. In general, NPs be able to synthesized by various approaches such as chemical, physical or biological method, depending on either the principle of bottom-up or top-down approach. The bottom-up approach fabricates NPs atom-atom or molecule - molecule by self-assembly or self-organization or cluster-cluster pairs [**4**]. In comparison to the conventional antibiotics, nanostructured antimicrobial drugs help in reducing the toxicity, preventing resistance, reducing the side effects of drugs and lowering the cost there for the pharmaceutical sciences are using nanoparticles [**5**].

This study aimed to investigate theantimicrobial effect of biosynthesized silver nanoparticles produced by *Streptomyces* spp. against different gram +ve and gram -ve tested bacteria.

Materials and methods

Study Design and *Streptomyces* spp. Isolates

This cross sectional study was designed to investigate theantimicrobial effect of biosynthesized silver nanoparticles produced by *Streptomyces* spp. against different gram +ve and gram -ve tested bacteria. At the beginning of this study, 75 different soil samples were collected from different geographical area in Babylon province, Iraq. All soil samples were cultured primarily on selective culture media specified for isolation of *Streptomyces* species such as soya bean agar and ISP2 agar medium. Out of 75 soil samples, 34*Streptomyces* spp.isolates were obtained. These isolates were identified as *Streptomyces* spp.Usingstandard microbiological methods according to Shirling and Gottlieb [**6**]. The identification of these isolates as *Streptomyces* spp were confirmed by amplification of 16sRNA gene using, 16sRNA gene primer, and assuggested previously [**7**].Two *Streptomyces* spp. isolates (*Streptomyces* spp.MU-43 and *Streptomyces* spp.SA-65)were selected for further investigations in the this study.

Biosynthesis of silver nanoparticles using *Streptomyces* spp. biomass

Silver nitrate ($AgNO_3$) was used as precursor for biosynthesis of silvernanoparticles by *Streptomyces* spp. Silver nitrate was added with concentration 5 mM (obtained by dissolved 0.85 mg of $AgNO_3$ in 100 ml of DDW) to biomass of *Streptomyces spp.(*MU-43 & SA-65*)*which distributed in sterilized flasks containingISP4 broth medium.This step was carried out under dark condition to avoid oxidation of $AgNO_3$.The pH of the reaction mixture was adjusted to 7. The resultant solutions were incubated in shaking incubator 200 rpm at 28° C for 7 days. The color change was observed After incubation. The reaction mixture was centrifuged at 10000 rpm for 10 minutes.The supernatant was discarded and replaced with deionized distill water and recentrifuged three times at the same speed and time to remove remained supernatant,the pellet deposit at bottom of tube that represent collection of nanoparticles then dried in oven at 40 °C for 24 hours. The dried powder was collected carefully and stored in sample vials for further analysis [**8-10**].

Antibacterial activity of biosynthesized AgNPs

Antibacterial activity of AgNPs biosynthesized by the two isolates of *Streptomyces* spp (MU-43and SA-65) was examined for their antimicrobial activity against different types of pathogenic bacteria isolated, including both Gram positive and Gram negative bacteria were determined by disc diffusion method using NO.1 Whatman filter paper [**11**]. V**olume of 30µlAgNPs a**nd concentration (5mM with 5 serial dilutions) was investigated by disc diffusion method to determine the concentration. From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up by sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of McFarland No. 0.5 turbidity standard. A sterile swab was dipped into the bacterial suspension; any excess

fluid was expressed against the side of the tube. The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows: The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.
The disc impregnated in **30 µ** of five serial dilution of AgNPs suspension. The plates were incubated at 37°C for 24 hours. After incubation, the plates were analyzed for the zones of inhibition. The activity was evaluated by calculating the increase in folded area.

Combination of antibiotics and Ag-NPs
The Combination between antibiotics-**AgNPs (30µl) against indicator** bacterial isolates were done by disc diffusion method. To determine the combination effect of antibiotics-AgNPs .The discs were impregnated against indicated bacterial isolates with prepared AgNPs and then these discs were used for antibacterial activity assays the plates were incubated at 37°C for 18-24 hours. After incubation, the plates were analyzed for the zones of inhibition. The activity was evaluated by calculating the increase in folded area.

Results

Antimicrobial activity of silver nanoparticles

Silver nanoparticles produced by two *Streptomyces* (MU-43& SA-65) were examined for the inhibition growth of bacterial isolates by disk diffusion agar method (Table 1).

Table (1): Antimicrobial activity for AgNP against tested bacteria

		Nanoparticles serial dilution (Mm)+ antibiotics				
Tested bacteria	***Streptomyces* spp NO.**	**1**	**2**	**3**	**4**	**5**
Klebsiella pneumonia	SM-43	21	18	18	16	16
	SM-65	17	16	15	14	14
Pseudomonas aeruginosa	SM-43	18	18	17	15	15
	SM-65	18	15	14	14	13
Staph. aureus	SM-43	16	16	15	14	11
	SM-65	13	13	12	11	9
Strep. Agalactiae	SM-43	15	14	12	11	10
	SM-65	14	13	13	12	9
Acinetobacter baumannii	SM-43	12	12	10	9	0
	SM-65	12	10	9	0	0

Inhibition of bacterial growth of tested isolates by silver nanoparticles fabricated by *Streptomyces* MU-43 was found to be more efficient when compared with that fabricated by *Streptomyces* SA-65 (Table 1). It's found that gram Negative bacteria showed high susceptibility to AgNPs fabricated by *Streptomyces* MU-43 compared with gram-positive tested bacterial isolates. The largest inhibition zone in *Staphylococcus aureus* produced by followed by *Strept. agalactiae*.On the other hand, among the studied gram negative bacteria, in case of AgNPs fabricated by *Streptomyces*MU-43,*K.pnemonia*e have been recorded to have the largest inhibition zone, followed by *P. aeruginosa* and *Acinetobacter baumanii*. Regarding silver nanoparticles fabricated by*Streptomyces* SA-65 strain, *P.aeruginosa* has been recorded to have the largest inhibition zone, followed by *K.pneumonia*e thesmallest inhibition zone recorded by *Acinetobacter baumanii*, *Strep. agalactia*e and *S. aureus* also recorded inhibition zones. Gram positive bacteria used in this study exhibited less susceptibility than gram negative to silver nanoparticles fabricated from two *Streptomyces* isolates (MU-43& SA-65).

Combination effect of antibiotic with AgNPs

Pathogenic bacteria isolates of *Ps. aeruginosa*, *A. bamanii*, *K. pneumonia, S. agalactiae* and *S. aureus*were tested for their antibiotic resistance against different types antibiotics using disk diffusion method. In this study, all tested isolates were resistant to minimum of 5 types of antibiotics within more than two different antibiotic classes under test, so the isolates were considered as multidrug resistant. Five MDR isolates (*Ps. aeruginosa*, *Acinetobacter baumanii*, *K. pneumoniae, S. agalactiae* and *S. aureus*) were subjected for antibacterial susceptibility with and without biosynthesized AgNPs using disc diffusion methods. Results found that the combination effect of antibiotic and silver nanoparticles by disc diffusion method gave increasing fold diameters of inhibition zone of bacterial isolates in comparison with antibiotic alone. The Combinations of antibiotics and AgNPs resulted in increasing in antibacterial activity (Tables 2-6).

Table (2): Effect of different antibiotics with and without biosynthesized AgNPs *against Staph. aureus*

Staph aureus			**Serial dilution+Antibiotics Nanoparticles (Mm)**					**Synergistic effect**
Antibiotic	**Inhibition zone(mm)**	***Streptomyces* pp. isolates**	**1**	**2**	**3**	**4**	**5**	
P	29	SM-43	33	28	27	25	23	_
		SM-65	23	23	22	19	17	_
AK	15	SM-43	24	23	21	19	19	_
		SM-65	20	20	19	17	16	_
CIP	22	SM-43	27	25	25	25	23	_
		SM-65	23	22	21	21	20	_
RA	13	SM-43	19	18	16	13	10	+
		SM-65	17	16	14	11	9	+
STX	0	SM-43	19	18	16	16	13	+
		SM-65	15	14	13	13	10	+
FOX	17	SM-43	30	25	22	21	19	+
		SM-65	20	19	19	16	12	+
DA	0	SM-43	22	21	20	21	18	+
		SM-65	13	12	12	10	10	_
F	20	SM-43	28	27	25	25	22	_
		SM-65	24	23	23	21	20	_
ATM	0	SM-43	25	25	24	23	21	+
		SM-65	21	20	20	19	19	+
C	0	SM-43	13	12	11	11	9	+

		SM-65	13	13	12	11	10	+
TE	21	SM-43	27	25	25	22	22	_
		SM-65	23	22	20	18	18	_

Penicillin P; Amikacin AK; Ciprofloxacin CIP; Rifampin RA; Trimethoprim-Sulphamethoxazole STX; Cefoxitin FOX; Clindamycin DA; Nitrofurantion F; Aztreonam ATM; Chloramphenicol C; Tetracycline TE.

The results revealed that *S. aureus* exhibited different degrees of sensitivity to antibiotics that used in the study.*Staph aureus* showed sensitive to P,Ak, Cip, Cefoxitin, F, TE, while they showed resistance to:RA, STX, DA,ATM,C. When mixed antibiotics with silver nanoparticles (in five serial dilutions) fabricated by *Streptomyces* MU-43, *S. aureus*did not show any resistance to any antibiotic (positive synergistic effect). The synergistic effect of silver nanoparticles synthesized from *Streptomyces* MU-43 represents the highest percentage of increasing in inhibition, which was found against P followed by STX and smallest inhibition zone represented by C. The combination the silver nanoparticles fabricated by *Streptomyces* MU-43 with each these antibiotics: RD, STX, DA, ATM and C were altered the resist of *Staphylococcus aureus* to sensitive for previous antibiotics. Although silver nanoparticles synthesized from *Streptomyces* SA-65 exhibitedinhibition the bacterial growth, but it's also revealed a lesser amount of efficacy than the *Streptomyces* MU-43, the combination the same antibiotics with silver nanoparticles that fabricated by *Streptomyces* SA-65 strain S.*aureus*stillresistant to DA and STX (negative synergistic effect). Largest inhibition zones were against F and smallest inhibition zone was againstC and DA.In addition to the fold increase area to the zones of inhibition with silver nanoparticles were reported TE, F, CIP, AK. Table (3) shows the susceptibility of*Strep. agalactiae* isolate to antibiotics used in this study. The results revealed the *Strep*t. *agalactiae* was resistant to P, RA, Cip, STX, DA, ATM and C and sensitive to TE, F, Fox, Ak. The results of conjugated antibiotics with silver nanoparticles biosynthesized by *Streptomyces* MU-43, revealed that positive synergistic effect of P, DA, ATM with AgNPs at which resistance altered to sensitivity but it was still resistant to C, STX and Cip. However, the inhibition zones to all antibiotics were increased regardless the susceptibility pattern of the isolates.

Table (3): Synergistic effect of different antibiotics with and without biosynthesized AgNPs against *Streptococcus agalactiae*

Streptococcusagalactiae			**Antibiotics + Serial dilution of nanoparticles**					**Synergistic effect**
AB	**Inhibition zone (mm)**	**Streptomyces spp. Isolates**	**1**	**2**	**3**	**4**	**5**	
P	0	SM-43	28	25	25	24	23	+
		SM-65	23	22	20	19	17	+
AK	20	SM-43	24	23	21	19	16	_
		SM-65	22	20	19	17	13	_
CIP	0	SM-43	18	15	14	11	9	_
		SM-65	16	13	13	10	10	_
RA	0	SM-43	19	17	16	14	11	+
		SM-65	18	16	13	11	9	_
STX	0	SM-43	16	15	15	14	10	_

		SM-65	16	14	13	11	9	_
FOX	16	SM-43	28	24	23	20	17	+
		SM-65	25	21	19	16	15	+
DA	0	SM-43	20	20	18	17	14	+
		SM-65	19	18	18	17	13	+
F	22	SM-43	29	27	24	24	19	_
		SM-65	24	24	22	21	19	_
ATM	15	SM-43	26	26	24	22	21	+
		SM-65	23	23	22	20	18	+
C	0	SM-43	14	13	12	10	9	_
		SM-65	14	12	12	11	11	_
TE	23	SM-43	24	22	20	19	19	_
		SM-65	21	19	19	17	16	_

+: antibiotics effect was changed from resistant to sensitive, _: antibiotics effect was still the same.

Regarding nanoparticles synthesized by *Streptomyces* SA-65, the largest inhibition zone was appeared in FOX while the smallest inhibition zone was in C. These antibiotics-AgNPs synergistic effect showed alteration of resistant *Streptococcus agalagtiae* isolates into sensitive against P, DA, ATM antibiotics but it was still resistantto C, STX and Cip. However, the inhibition zones to all antibiotics were increased regardless the susceptibility pattern of the isolates. Table (4) illustrates the susceptibility of *A. baumannii* isolate to antibiotics. The results found that this isolate was resistant to all antibiotics tested in this study. The antibiotic–AgNP conjugate showed inhibition of *A. baumannii* growth in different degrees. The largest inhibition zone resulted from synergism of antibiotics-nanoparticles synthesized by *Streptomyces* MU-43 was seen in CTX, while the smallest inhibition zone was appeared in Chloramphenicol. Regarding the *Streptomyces* SA-65, the largest inhibition zone was seen in MEM while the smallest inhibition zone was seen in C.

Table (4): Synergistic effect of different antibiotics with and without biosynthesized AgNPs against *Acinetobacter baumannii*

Acinetobacter baumannii			**Serial dilution nanoparticles +antibiotics**					**Synergistic effect**
AB	**Inhibition zone (mm)**	**Streptomyces spp isolates**	**1**	**2**	**3**	**4**	**5**	
AMC	0	SM-43	19	18	17	15	13	+
		SM-65	19	18	16	15	15	+
ATM	0	SM-43	22	21	19	16	15	+
		SM-65	20	18	17	17	14	+
CIP	0	SM-43	27	27	24	22	20	+
		SM-65	24	22	19	16	14	+
		SM-43	26	23	22	21	21	+

MEM	0							
		SM-65	26	23	21	20	19	+
STX	0	SM-43	19	18	15	14	11	+
		SM-65	17	14	12	10	9	+
CTX	0	SM-43	28	26	25	22	20	+
		SM-65	25	22	19	16	15	+
APX	0	SM-43	20	19	17	17	14	+
		SM-65	19	18	18	16	13	+
F	0	SM-43	22	20	19	17	16	+
		SM-65	20	17	15	12	9	+
AK	0	SM-43	21	20	18	18	15	+
		SM-65	19	19	17	15	14	+
C	0	SM-43	16	15	13	10	9	_
		SM-65	15	13	12	11	10	_
TE	0	SM-43	25	22	21	18	17	+
		SM-65	23	21	20	19	17	+

Acinetobacterbuomanii exhibited resistance against C until after impregnated in silver nanoparticles biosynthesized by both *Streptomyces* strain (MU-43 & SA-65). Using garlic (*Allium sativum*) and sodium citrate to synthesized silver nanoparticles combination of Chloramphenicol - AgNP also showed better synergistic activity.

Pseudomonas aeruginosa (isolated from burn patients) has also been examined for antibiotics susceptibility. The results revealed that *P. aeruginosa* was susceptible toSTX, MEM, andTE but it was resistant to APX,CTX, CIP,AMC, ATM, F, AK, C (Table 5). The antibiotic–AgNP conjugate showed alteration of resistant *P. aeruginosa* isolates into sensitive for some antibiotics.

Table (5): Synergistic effect of different antibiotics with and without biosynthesized AgNPsagainst *Pseudomonas aeroginosa*

Pseudomonas aeruginosa			Antibiotics + Serial dilutionnanoparticles					
AB	**Inhibition zone (mm)**	***Streptomyces* spp. isolates**	**1**	**2**	**3**	**4**	**5**	**Synergistic effect**
AMC	0	SM-43	28	25	25	23	20	+
		SM-65	25	24	22	21	19	+
ATM	0	SM-43	26	23	21	20	18	+
		SM-65	24	23	23	21	19	+
CIP	0	SM-43	32	29	27	27	25	+
		SM-65	24	23	23	22	22	+
MEM	19	SM-43	31	29	25	23	20	_
		SM-65	27	26	25	22	22	_
STX	17	SM-43	19	17	17	15	15	_
		SM-65	19	19	18	16	16	_
CTX	0	SM-43	32	29	28	28	27	+
		SM-65	27	26	26	23	21	+
APX	0	SM-43	25	22	18	17	16	+
		SM-65	20	18	17	16	13	+
F	0	SM-43	14	14	13	13	10	_
		SM-65	14	13	13	12	10	_
AK	0	SM-43	17	17	15	14	12	+
		SM-65	13	13	12	10	10	+
C	0	SM-43	16	14	13	10	10	_
		SM-65	12	11	11	10	10	_
TE	15	SM-43	27	25	21	19	17	_
		SM-65	23	21	19	19	15	_

The largest inhibition zone observed after combination the antibiotic-silver nanoparticles biosynthesized by *Streptomyces* MU-43 was found in CIP and CTX and the smallest inhibition zone found in Chloramphenicol, whereas thelargest inhibition zones result from synergism the antibiotics-silver nanoparticles fabricated by *Streptomyces* SA-65 strain were observed in MEM CTX and smallest inhibition zone in C.

Table (6): Synergistic effect of different antibiotics with and without biosynthesized AgNPs against *Klebsiella pneumonia*e

Klebsiella pneumoniae			**Serial dilutionnanoparticles +antibiotics**					**Synergistic effect**
AB	**Inhibition zone(mm)**	***Streptomyces* spp isolates**	**1**	**2**	**3**	**4**	**5**	
AMC	18	SM-43	26	24	24	23	21	_
		SM-65	22	21	18	18	15	_
ATM	25	SM-43	31	30	29	27	26	_
		SM-65	28	25	23	22	21	_
CIP	19	SM-43	39	38	38	36	35	+
		SM-65	34	33	31	30	28	+
MEM	25	SM-43	33	31	29	27	26	_
		SM-65	30	28	26	25	25	_
STX	0	SM-43	22	22	20	20	19	+
		SM-65	22	21	19	19	17	+
CTX	28	SM-43	36	33	33	31	27	_
		SM-65	30	27	26	23	20	_
APX	0	SM-43	25	22	18	17	16	+
		SM-65	20	18	17	16	13	+
F	0	SM-43	19	17	17	16	13	+
		SM-65	19	13	13	12	10	+
AK	0	SM-43	23	22	21	19	17	+
		SM-65	23	22	20	18	16	+
C	0	SM-43	14	14	13	12	10	+
		SM-65	12	12	11	10	9	+
TE	19	SM-43	25	25	23	22	19	_
		SM-65	23	21	19	17	16	_

Regarding to tested *K.pneumonia*e isolates, theywere sensitive to ATM, AMC,CTX MEM, TE and resistant to STX,APX,F, AK,C,CIP(Table 6). The present study revealed that the lowest synergism effect have been found in Chloramphenicol against all tested bacteria (Tables 2, 3, 4, 5, and 6).In the present study, silver nanoparticles were diluted by distilled water, this dilution have significant effect on antimicrobial activity of silver nanoparticles alone and also when conjugated with different antibiotics. The result revealed that first concentration (100%) gave the highest inhibition zones (Tables 1, 2, 3, 4, 5, and 6).

Discussion

Differential susceptibility to sliver nanoparticles among gram positive and gram negative bacteria may be attribute to the structural differences between gram positive and gram negative cell envelop (Thicker Pg in G+ve bacteria) and quorum sensing [**12]**. Differential susceptibility among gram positive or gram negative members might be attributed to variety in the inherent resistome i.e. (efflux and other resistance machinery, presence and strength of silver resistance plasmid containing *sil* gene) [**13**]. Kaviya*et al.*[**14]**reported that silver nanoparticles biosynthesized by *Streptomyces* spp. exhibited good antibacterial activity against both gram negative and gram positive bacteria, they showed higher antibacterial activity against and *P.aeroginosa* (Gram negative) than *S. aureus* (Gram positive).Antibacterial activity against tested bacteria was observed to be different regarding the source of biogenic AgNPs applied. The results showed that gram negative bacteria were higher sensitive than gram positive bacteria to AgNPs. Furthermore AgNPs fabricated by *Streptomyc*es MU-43 isolate had higher activity than AgNPs fabricated by *Streptomyces* SA-65 isolate. Differential antimicrobial activity among AgNPs fabricated by different bacteria (even strains from the same species) attributed to the difference in the capping molecules, and the physical characteristics of the nanoparticles like size, shape and dispersity [**15]**. The mechanism behind the bactericidal effect of the silver nanoparticles against bacteria is not well known. It is believed that DNA loses its replication capacity and cellular proteins become inactivated upon silver ion treatment in addition, higher concentrations of Ag+ ions have been revealed to interact with cytoplasmic components and nucleic acids [**16**]. The major reason why nanoparticles is considered an alternative to antibiotics is that nanoparticles can successfully avoid microbial drug resistance in some cases because the bacteria have powerful ability of developing resistance against antibiotics. The extensive use of antibiotics has led to emergence of numerous hazards to public health, such as superbugs that do not respond to any existing drug and non-medicated epidemics. [**17**]. We can conclude the mechanism behind the bactericidal effect of the AgNPs;the first mechanisms, the impact may be due to ultrafine size for the AgNPs and the larger surface region, while their positively charged Ag+ ions attach to the negatively charged which present in bacterial cell wall, leading to deactivating the cellular enzymes, therefore causing disruptions in the membrane permeability [**18**]. The second, AgNPs via interactions with the thiol group of L-cysteine protein residues will lead to enzymatic dysfunction **[19]**.Finally, the silver nanoparticles causes damage on proteins and DNA via release of reactive oxygen species (ROS) [**20**]. Zarina and Nanda[**21**] used *Streptomyces albaduncus*to biosynthesize silver nanoparticles and they estimated its antibacterial activity. They found that synergistic effect of different antibiotics with and without extracellularly biosynthesized AgNPs against *S. aureus, Pseudomonas* spp*, E. coli, K.pneumoniae, Micrococcus luteus, Strept. mutans* were increased. The bonding reaction between silvers nanoparticles and antibiotic may causes increasing the synergistic result.

Silver nanoparticles have ability to anchor to bacterial cell wall and subsequently penetrate it, thereby causing structural changes in cell membrane like the permeability of the cell membrane and death of cell. There is a formation of **'**pits**'** on cell surface, and there is accumulation of the nanoparticles on the cell surface [**22**]. In synergism, the bactericidal effect is enhanced by interaction between active groups like hydroxyl and amino groups present in these antibiotics with AgNPs by chelating. As a outcome, antibiotic–AgNP conjugate is formed in which an AgNP core is surrounded by antibiotic molecules. Thus, the antimicrobial concentration is enlarged at the focal site, which leads to increased destruction of bacteria [**23**]. Synergism among antibiotics-silver nanoparticles biosynthesized by both *Streptomyces* strain (MU-43 & SA-65) showed that this synergism was the most efficient to inhibit *Klebsiellapneumonia*e growth. This effect may be come from either increasing the drug bio-availability after conjugation in the cell membrane of bacteria or may be due to assimilatory effect of both components. Therefore, the use of AgNPs in the association with antibiotic showed synergistic effect **[24**]. Abod*et al.*[**25**] studied the antimicrobial activity of silver nanoparticles biosynthesized by *Streptomyces*. spp against some pathogenic bacteria and they found

that Chloramphenicol, Tetracycline, and Doxycycline when conjugated with silver nanoparticles showed the lowest synergism effect against all tested bacteria. The largest inhibition zone was shown in gram negative in comparison with gram positive bacteria. This difference was possibly attributed to the difference of the peptidoglycan layer of the bacterial cell between G+ve and G-ve bacteria (Tables 2, 3, 4, 5, and 6).The Gram negative cell envelope consists of outer membrane, thin peptidoglycan layer, and cell membrane, while in the Gram positive the cell envelope consists of lipoteichoic acid containing thick peptidoglycan layer and cell membrane [**26**]. Multidrug resistance (MDR) has been identified as a main threat to the public health of human being by the World Health Organization [**27**].These bacteria significantly compact the efficacy of antibiotic, consequently, increasing the frequency of therapeutic failure and mortality. This rising antimicrobial resistance has been accompanied by a decline in new antibiotic discovery over the last few decades, which now poses a serious threat to public health [**28**]. There are variances in the degree of sensitivity of tested pathogenic bacteria to silver nanoparticles when exposed to the same concentration. This may be due to the differences in intrinsic susceptibility of bacterial species depends on the concentrated activity of several elements, what has been named as intrinsic resistome i.e. (efflux and other resistance machinery, presence and strength of silver resistance plasmid containing *sil* gene) **[29].** It has been suggested that silver nanoparticles interfere with bacterial replication processes by adhering to their nucleic acids. The interaction of silver ions with sulfhydryl (SH) groups of proteins that cause the DNA unwinding, and contact with hydrogen bonding processes are also been demonstrated lead to cell division was inhibited [**30**].The silver ions is known to mainly inhibit enzymes such as NADH dehydrogenase II in the respiratory system, which is involved as a candidate for the site of production of reactive oxygen species [**31]**. The ribosomes may be damaged by silver ions or small AgNPs as a consequence inhibition of protein synthesis as well as translation and transcription can be prevent by the binding the AgNPs with the genetic material of the bacterial cell, [**32**]. It has also been found that the nanoparticles can modulate the signal transduction in bacteria by dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth [**33]**. Results of this study also found that concentration of silver nanoparticles has an important role in increasing the inhibition growth of tested pathogenic bacteria. Silver nanoparticles were diluted by distilled water with increasing the volume of nanoparticles solution. In most of the research experimental conditions, Water, which is considered as a **"**Universal solvent **[34**]?

Organisms that inherent *sil* gene (silver resistance machinery) can synthesize silver nanoparticles indicating that each microorganism has its own threshold limit of AgNPs concentration. The resistance mechanism that the microorganism follow to override threshold limit of AgNPs concentration varies from one microorganism to another. Extracts from bio-organisms may act both as reducing and capping agents in AgNPs synthesis. Organisms which contain the **"**Silver resistance machinery**"** can synthesize silver nanoparticles provided that the concentration of the silver ions does not cross the **"**threshold limit**"**. The resistance mechanism varies with organisms. Extracts from bio-organisms may act both as reducing and capping agents in AgNPs synthesis [**35**].

Conclusions

AgNPs Biosynthesized by *Streptomyces* spp. have antimicrobial activity against both gram positive and gram negative bacteria. Gram negative bacteria were more susceptible to biogenic silver nanoparticles biosynthesized by *Streptomyces* spp. AgNPs fabricated by *Streptomyc*es MU-43 isolate had higher antimicrobial activity than that AgNPs fabricated by *Streptomyces* SA-65 isolate.

Conflict of interest

None of the authors have any conflicts of interest relevant to this research subject.

Ethical Approval

Ethical Committee at the University of Babylon, college of Medicine, approved the study.

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