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RESEARCH ARTICLE

The Effect of Silver and Zinc Oxide Nanoparticles on Multi Drug Resistance *Staphylococcus aureus*

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Abstract

Forty two isolates of *Staphylococcus aureus* were isolated from different clinical sources distributed among 24(57.2%) isolates from Urinary tract infection, 8(19.0%) isolates from wound and 10(23.8%) isolates from pus for the patients attending the laboratories of Rizgary Teaching Hospital and Rizhawa Hospital in Erbil City from March to September 2013.The isolates were diagnosed based on cultural characteristics, microscopical features and biochemical tests in addition to the API Staph. The study accomplished with sensitivity of isolates against (12) types of antibiotics. Eighty eight percentage of the isolates were resistant to Ampicillin (AM/10 µg), 81% resistant to Amoxicillin (AX/25 µg) while the lower resistance was registries against Nitrofurantoin (NIT/300 µg), Ciprofloxacillin (CIP/5 µg) and Gentamicin (GM/10 µg) 21.4%, 26%, 31% respectively**.** Ten isolates were selected according to their pattern of the highest resistance as these showing multi-drug resistances and tested to specify their minimum inhibitory concentration (MIC) for the antibiotics and two types of Nanoparticles include Silver in different sizes (20, 90)nm and Zinc Oxide in different sizes (20, 30, 50~150)nm. The results showed that the MIC for Ag20nm was between (650 -2600) µg/ml and Ag90nm was between (325 -2600) µg/ml while the MIC for Zn O20, 30, 50~150nm was between (325-2600) µg/ml for different sizes. Synergism effect between the antibiotics and the Nanoparticles when they integrate increased their effect of *Staphylococcus aureus.*

The AMPc gene was founded in six isolates under study where extent in191bp but the bla_{CTX} gene wasn't founded in this isolates where extent in 701 bp. Scanning electron microscopy (SEM) was used to study the biocide action of this Nanoparticles. The results confirmed that the treated Staph.aureus cells were damaged.

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Introduction

Staphylococcus aureus is one of the most common human pathogens, especially in hospital patients. This gram positive bacterium is responsible for various infectious diseases, and has a great capacity to develop resistance to antimicrobial agents (1).

Nanotechnology has attracted global attention because Nanoparticles (NP) have properties unique from their bulk equivalents. NP of Ag, CuO and ZnO are being used industrially for several purposes including amendments to textiles, cosmetics, sprays, plastics and paints (2). A common feature of these three NP is their antimicrobial activity (3). Because of the quantum size effect of Nanoparticles that is different from the bulk,

Nanoparticles' physical and chemical properties qualified them to be used in much application in the electronic, chemical and mechanical industries, drug carriers, sensors, magnetic and electronic materials (4).

Nanoparticles usually ranging in dimension from1-100 nanometers (nm). With the decrease in the dimensions of the materials to the atomic level, their properties change .Metal Nanoparticles with antimicrobial activity when embedded and coated on to surfaces can find immense applications in water treatment, synthetic textiles, biomedical and surgical devices, food processing and packaging (5).It has been known that silver and its compounds have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities for bacteria, fungi, and virus since ancient times (6,7).

Nanoparticles as antimicrobial agents is their better efficiency on resistant bacteria, less toxicity and heat resistance, and among metal oxide Nanoparticles, ZnO Nanoparticles have many significant features such as chemical and physical stability, high catalysis activity and effective antibacterial activity(8). The antibacterial activity of ZnO NPs against many isolates has been well documented like in *Staphylococcus* spp. (9), *K. pneumoniae* (10), and *Pseudomonas* spp. (11), *Escherichia coli* (12).

Recently, the combination of the silver Nanoparticles with different antibiotics was investigated for activity against *Staph .aureus* and *E. coli*. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased in the presence of silver Nanoparticles against both tested bacterial strains (13).

This study was conducted in order to investigate the prevalence of multi-resistance of *Staph.aureus* to antibiotics that causes many diseases and to assess the impact of Nanoparticles on bacteria and used as alternative or as assistance to antibiotic in treatment.

Materials and Methods

Isolation and Identification of Staphylococcus aureus isolates

One hundred fifty six samples were collected from different human infection(Urinary tract infection, wound, pus) from Rizgary and Rizhawa Hospital in Erbil City Since March to September 2013.

The specimens were inoculated on the Nutrient agar and incubated at 37ºC for 24 hours. The isolates were examined for their shape, size and color, then transferred and streaked on manitol salt agar which considered as selective and differential medium for the isolation, purification and identification of Staphylococci, and for detecting the ability of each isolate to ferment manitol. All plates were incubated at 37ºC for 24 hours then a single pure isolated colony was transferred to Nutrient agar for the preservation and to carry out other biochemical tests that confirmed the identification of isolates (14,15) also API Staph was used for diagnosis.

Detection of Susceptibility to Antibacterial Agents: Susceptibility of all the isolates to different antibiotics were determined by the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute CLSI (16).The antibiotic discs used in this study were Ampicillin (AM/10 µg), Amoxicillin(AX/25 µg),Gentamicin(GM/10 µg), Erythromycin(E/15 µg), Ciprofloxacillin(CIP/5 µg), Chloramphenicol(C/30 µg), Cefotaxime(CTX/30 µg), Nitrofurantoin(NIT/300 µg), Co-Trimoxazole(COT/25 µg), Cephalothin(KF/30 µg), Doxycyclin(DO/30 µg), Ceftriaxone(CRO/30 µg)**.** Each antibiotic concentration was applied on the surface of Muller -Hinton agar plates and inoculated with Staph.aureus isolates and incubated at 37°C for 24 h. Staph.aureus ATCC 25923 used as quality controls (17).

Minimum Inhibitory Concentration (MIC) of Antibiotic and Nanoparticles against *Staph.aureus* isolates

Stock solutions of Antibiotic and Nanoparticles

Preparation of stock solutions was carried out by weighting the antibiotic powders and dissolved in Mueller-Hinton broth. The highest desired concentration was 1024µg/ml, therefore two fold dilutions were done, the stock solution diluted to (512µg/ml). All stocks of antibiotic solutions were kept on ice until use.

Commercially synthesized Ag and ZnO NPs were purchased from M K Impex Corp., CANADA. The reported ''as manufactured'' sizes were: AgNPs\20, 90 nm and ZnO NPs \20,30,50~150 nm. Preparation Stock solution according to the method (18) add 100mg of nanoparticles to 10ml of Deionizer water and requested vigorously for 5 minutes to break the bloc and get a homogeneous solution and then sterilized degree 121 C for 20 minutes and cool in temperature room for a final concentration of stocks 10mg / ml.

Inoculums preparation

The 18 hrs cultures plate from all *Staph.aureus* isolates were prepared. Single colonies from each isolated plate were transferred to 5 ml sterile suspension media to obtain 10^6cfu/ml, which were also adjusted with 0.5 McFarland tube.

MIC for Antibiotic and Nanoparticles

Ninety six flat well microtiter plates were used for determination of Bacteriostatic activity of antibiotics and nanoparticles according to the methods described by (19):-

•100 µl of Mueller-Hinton broth was dispense into all wells of a microtitre plate.

•100 µl of type one antibiotic stock solution 1024µg/ml was transferred into the well 1 (far left of plate) in row A. The antibiotic was mixed into the well 1 by sucking up and down 6-8 times. This makes well 1 a twofold dilution of stock (i.e. 512µg/ml).

•100 µl was withdrawn from well 1 and added to well 2 in the same row.

This makes well 2 a twofold dilution of well 1 (i.e.256µg/ml). This procedure was repeated down to well 10 only and the concentrations (512,256,128,64,32,16,8,4,2,1) µg/ml and100 µl from well 10 was discarded rather than putting it in well 11 continue only broth and bacterial suspension (kept as positive control of the test).

•The same set of tips was used for the remained types of antibiotics in different rows with same plate.

•To prevent cross-contamination, one type of bacterial isolate with different types of antibiotic was used in the same plate.

The plate inoculation was carried out by adding 5 µl of bacterial suspension into wells in columns 11 to 1 but not column 12 (control negative) and then all plates were incubated at 37C^{o} for 24 hrs.

After incubation, the MIC was determined as described previously.

As for the concentration of nanoparticles have been used 5200 µg/ml was transferred into the well 1 in row A. the concentrations were (2600, 1300, 650,325,162.5, 81.25, 40.6, 20.3, 10.15, 5.07) µg/ml.

Determination of antimicrobial activity and Synergistic effect of silver and zinc oxide nanoparticles with antibiotics by well-diffusion method

The antibiotics ,ZnO and Ag NPs were tested for antimicrobial activity by well-diffusion method against *Staph.aureus* isolates ,which were making 5 wells of 6-mm diameter were made on Mueller–Hinton agar plates using gel puncture and add 100 µL of (MIC) for antibiotics and nanoparticles for 1,2,3 wells and 4 well were added 50 µL of (MIC) for nanoparticles with 50 µL of (MIC) for antibiotic to study the synergistic effect of silver and zinc oxide with antibiotics, while added distilled water to 5 well for control and incubated at 37°C for 24 h. Each plate was examined and measured for the diameters of inhibition zones including the diameter of the wells also.

The use of infrared spectroscopy to diagnose nanoparticles correlation with antibiotics

IR spectra were used FTIR-600, Biotech engineering Management Co. LtD .UK for the diagnosis of chemical bonds (effective aggregates) in the antibiotics and their interaction with nanoparticles. The nanoparticles with antibiotics were pressed using potassium bromide (KBr) and then measured samples by IR at wavelength spectrum 400-4000cm-1.

DNA extraction:

A single colony of cultivated bacteria, which had been incubated overnight, suspended into 1ml of distilled water, centrifuged at 14000xg for 2 min., then the supernatant discarded, after that 120µL of lysostaphin (10 mg/L; Sigma) was added. DNA extracted using mini DNA extraction kit (Promega) according to manufacture instructions.

PCR amplification procedure:

Specific primers were designed for amplification of *AMPc* & bla_{CTX} gene Table (1).These primer synthesized by Alpha DNA Company, Canada.

PCR reaction was conducted in 25µl of a reaction mixture containing 4µl of DNA, 12.5 µl Go Taq Green Master (Promega, CA), (0.5 µl) 25mM MgClR2R, 1µl of (10 Pmol\ µl) of each primer and 6.5 µl of Nuclease free Water. Amplification program for , *AMPc* gene the reactions mixtures Depending on the(20) included an initial denaturation at 94°C for 1.5 min consisted of 30 cycles of 94 ºC for 90s, ,specific annealing temperature 57◦ C for 30s , 72 ºC for 60s and a final cycle of primer extension at 72°C for (10) min in a Thermal Cycler. Whereas for bla_{CTX} the reactions mixtures Depending on the(21) included an initial denaturation at 94°C for 30 sec. specific annealing temperature 50 ºC for 30 sec. and 72 ºC for 90 sec., and a final cycle of primer extension at 72°C for 7 min. The amplified product was subjected to 1.5% agarose gel electrophoresis, and visualized under UV after ethidium bromide staining.

Table (1):Primer used for the amplification of *AMPc* & *bla*$_{CTX}$ gene.

Primers	Sequence(5'→3')	Length	Amplification product(bp)
AMPc-F	AATGGGTTTTCTACGGTCTG	20	191
AMPc-R	GGGCAGCAAATGTGGAGCAA	20	
bla$_{CTX}$-F	AATCACTGCGTCAGTTCAC	19	701
bla$_{CTX}$-R	TTTATCCCCCACAACCCAG	19	

Infrared spectroscopy to diagnose nanoparticles association with antibiotics

Spectrometer infrared were used IR-Spectrophotometer type FTIR-84005-Fourier Transform to diagnose the chemical bonds (effective groups) in antibiotics and their interaction with nanoparticles as pressure nanoparticles with antibiotics using potassium bromide and then measured at wavy 400-4000cm^{-1}.

Scanning electron microscope (SEM)

Sample preparation: It is performed according to (22, 23) as follows:-

1. 100µl of Staph.aureus suspension which prepared from subMIC of each different nanoparticals were fixed with 100µl formalin 10% in phosphate buffer (PH 7.4) for 10 min.
2. A Loop full of the mixture (1) were mounted on aluminum stubs or glass slides and allowed to dry for 40 min.
3. Stubs were then coated with pure gold by sputter coater.
4. Each stub was placed on the stage of SEM and about 5 random SEM fields, at high magnification were examined and images were captured.

Sample reading:

After preparation of exposed isolates, they were mounted on a glass slide (1cm X 1cm), and exhibited through the screen SEM by mechanical and computerized techniques when selected the pictures for further interpretation.

Results & Discussion

Figure (1) show that from total 156 clinical samples 42(26.9%) *Staph.aureus* were isolated which based on morphological characteristics, biochemical tests and API Staph. 24(57.2%) isolates were from Urinary tract infection (UTI), 8(19%) from wound infection and 10(23.8%) isolates from Pus.

Staph.aureus known to be a cause of UTI among catheterized patients (24).While (25) found that the percentage of Staph.aureus isolates in 301 urine samples was (13%) in Al-Hilla / Iraq. The variation may be differences in personal hygiene, environmental conditions and health status.

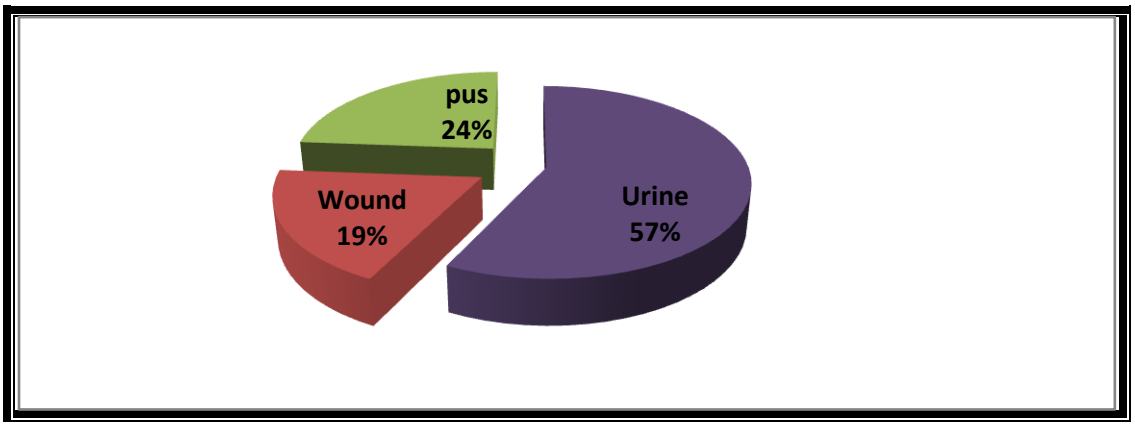

Figure (1) Proportions of *Staphylococcus aureus* isolated from different sources

Staph.aureus isolated were identified using traditional morphological and biochemical diagnostic tests according to the (26) and was in agreement as shown in (Table 2).

Table (2) Morphological and biochemical tests for identification of *Staph.aureus*

Test	Result	%	Test	Result	%
Gram stain	+	100	Indole	+	100
Catalase	+	100	Manitol fermentation	+	97.6
Oxidase	-	100	Citrate	-	100
Coagulase	+	100	Urease	+	92.8
DNase	+	95.2	Voges proskour	-	95.2

The conformational identification of Staph.aureus was performed using API Staph system.

Figure (2) represents antibiotic sensitivity pattern for 42 Staph.aureus isolates. The *Staph.aureus* isolates that recovered from clinical samples were highly resistant to most antibiotics that used in present study. The study accomplished with sensitivity of isolates against (12) types of antibiotics.88% of the isolates were resistant to Ampicillin,81% resistant to Amoxcilin while the lower resistance was registries against Nitrofurantoin , Ciprofloxacillin and Gentamicin (21.4,26,31) % respectively..

The mechanism of this resistance is mostly due to either production of β-lactamase that hydrolyze β-lactam ring which is controlled by plasmid or chromosomal regulation, or lack of penicillins receptors on cell wall and/or alteration in their permeability to β-lactam antibiotics preventing the up taking of them (27).This can be attributed to the fact that, antibiotics may have revolutionized the treatment of common bacterial infections (28) and some isolates have virulence factors more than other isolates, also differences in source of samples, conditions of tests used and type of techniques. All these factors may lead to differences in resistance levels (29).

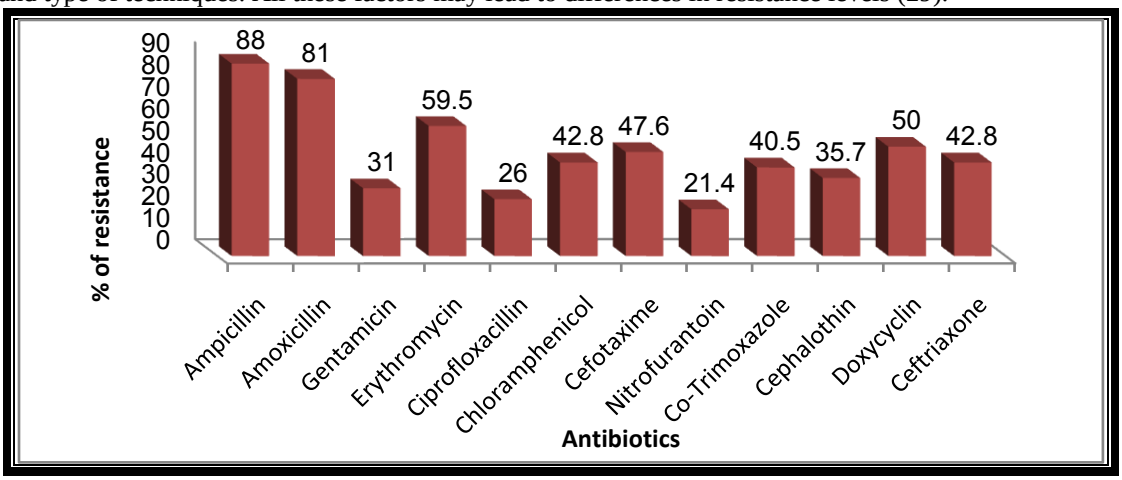

Figure (2): Represents antibiotic sensitivity pattern for 42 isolates belongs to *Staphylococcus aureus*

Determined minimum inhibitory concentration MIC of antibiotics studied where adopted on Break point described by (30, 16) as the basis for calculating the response when comparative with Staph.aureus ATCC25923. Results in the table (3) showed that MIC values of all *Staph.aureus* isolates were resistant to Ampicillin, Amoxicillin and Co-Trimoxazole as values ranged between (8-512) µg/ml while the MIC values for Gentamicin and Erythromycin were ranged between (16-512) µg/ml. As for Doxycyclin was able to 3 isolates out of 10 isolates resistance to antibiotic concentration (16-64) µg/ml and 5 isolates resistance against Chloramphenicol it values ranged between (32-64) µg /ml. As for Cefotaxime was able to 4 isolates out of 10 isolates resistance to antibiotic concentration (64-512) µg/ml and 7 isolates resistance against Cephalothin it values ranged between (32-256) µg /ml while all Staph.aureus isolates show sensitivity to Nitrofurantoin and Ceftriaxone except 1 isolate show resistance concentration 512 µg/ml.

Table (3):MIC value for 12 antimicrobials (µg /ml) against Staph.aureus isolates

Isolates / Antibiotics	*Break points	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	*Staph. aureus* ATCC25 923
	MIC(µg/ml)											
Ampicillin	≥0.5	128	128	512	32	64	128	64	128	8	128	-
Amoxicillin	≥0.5	512	128	64	64	32	512	128	256	16	32	0.25
Gentamicin	≥16	512	64	64	32	32	128	256	32	16	16	0.25
Erythromycin	≥8	512	128	64	32	64	256	128	512	16	32	0.5
Ciprofloxacillin	≥4	8	16	2	4	32	16	4	4	32	32	0.5
Chloramphenicol	≥32	64	8	32	32	8	32	8	1	1	32	-
Cefotaxime	≥64	512	32	128	32	64	8	16	32	4	128	-
Nitrofurantoin	≥128	32	4	16	4	8	8	1	16	8	32	-
Co-Trimoxazole	≥4	512	512	128	128	512	256	64	128	512	256	-
Cephalothin	≥32	16	8	64	32	128	32	16	128	32	256	-
Doxycyclin	≥16	2	16	1	1	2	64	16	2	2	1	-
Ceftriaxone	≥64	512	32	2	8	4	16	8	2	4	4	-

S: strain , *Standard breakpoints MIC of antibiotic (CLSI, 2011).

Table (4) showed that the MIC for Ag20nm was between (650 -2600) µg/ml and Ag90nm was between (325 -2600) µg/ml the antibacterial activities of Ag-NPs against Staph.aureus by formation of ROS induced by Ag-NPs (31). The MIC for Zn O20, 30, 50~150nm was between (325-2600) µg/ml. While the MIC for Zn O30, 50~150nm was between (162.5-2600) µg/ml .The effect of ZnO nanoparticles with the cells and increased production of active oxygen such as H2O2, leads to the cell death (32).

Table (4): MIC value for Ag & ZnO nanoparticals (µg /ml) against Staph.aureus isolates

Isolates / NP	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
	MIC (µg/ml)									
Ag20nm	650	1300	2600	2600	2600	650	650	1300	1300	1300
Ag90nm	325	1300	2600	650	1300	2600	2600	1300	2600	1300
ZnO20nm	2600	1300	650	650	325	1300	1300	650	2600	1300
ZnO30nm	162.5	650	325	1300	2600	650	650	325	2600	1300
ZnO50~150nm	650	325	650	1300	2600	1300	1300	162.5	2600	2600

Table (5) and figure (3) shows the Synergistic effect between antibiotics used in the study and Ag and ZnO nanoparticles when they mixed together using well diffusion method .The results showed that the rate of inhibition zone diameter increased when mixing antibiotics with nanoparticles in different sizes (Ag20, 90nm, ZnO20, 30, 50 ~ 150nm) .

Table (5) Synergistic effect of nanoparticles with antibiotics on *Staph.aureus* isolates

Antimicrobial agents	Effect of antimicrobial agents alone	Effect of antimicrobial agents +Ag20nm	Effect of antimicrobial agents +Ag90nm	Effect of antimicrobial agents +ZnO20	Effect of antimicrobial agents +ZnO30	Effect of antimicrobial agents +ZnO50	Effect of Ag20nm alone	Effect of Ag90nm alone	Effect of ZnO20nm alone	Effect of ZnO30nm alone	Effect of ZnO50nm alone
	mm										
Amp	0	13	12	14	13	15	8	0	11	8	10
AX	0	10	9	13	12	12					
GM	12	18	16	19	17	15					
E	0	20	22	20	21	22					
CIP	20	25	23	19	17	16					
C	10	17	16	17	16	17					
CTX	14	20	19	20	17	20					
Nit	15	20	17	18	18	15					
CoT	0	12	11	10	10	12					
KF	11	20	18	23	22	20					
Dox	13	15	16	15	18	11					
CRO	12	15	13	20	20	18					

Our results agreed with studies of other researchers (33, 34 and 35) which they concluded that synergistically between nanosilver with a number of antibiotics against E. coli and *Staph.aureus* which increased sensitivity against antibiotics. In our experiment we concluded that the synergy between nanoparticles and antibiotics increases Inhibitory effect of anti-bacterial, as in bacteria Staph.aureus.

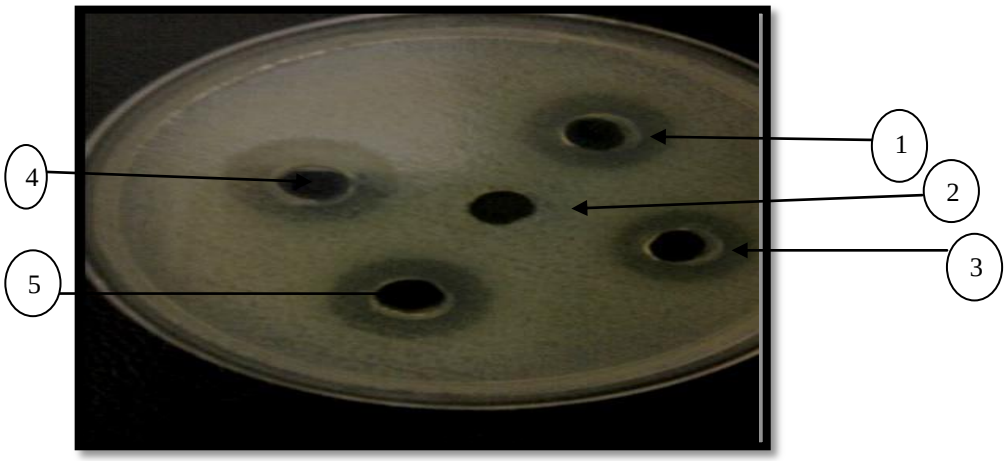

Figure (3) the Synergism effect of nanoparticles & antibiotics against Staph.aureus isolates
1: ZnO-NP Alone, 2: Control, 3: Ag-NP20nm alone, 4: Ag-NP20nm& Gentamicin, 5: Gentamicin

Amplified AMPc gene for *Staph. aureus* isolated showed resistant strains contain this gene by observing the band site and weight molecular at the extent of 191bp compared with the DNA Ladder as shown in the Figure (4) This is referred by (36)

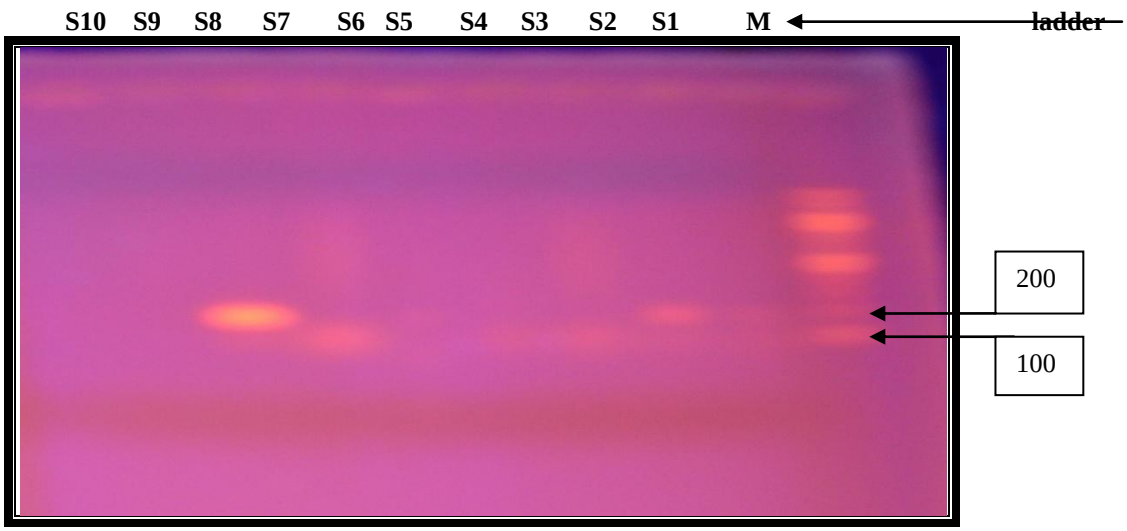

Figure (4) the band site at the extent of *AMPc* gene in 191bp for *Staph.aureus* strains.

Show Table (6) the number of isolates where resistance for *AMPc* & bla_{CTX} gene. The *AMPc* gene was founded in six isolates under study where extent in191 bp but the bla_{CTX} gene wasn't founded in this isolates where extent in 701 bp. Resistance to Ampicillin but not possess the *AMPc* gene may be Resistance may be caused by another gene belonging to the same ESBL group has not been investigating him in our study, encodes for the production of enzymes (ESBL) by genes located on large plasmids, although these plasmids easily transmitted between different types of bacteria and combine resistance genes result in strains possess multiple resistance plasmids, and for this reason, the isolates producing these enzymes are resistant to different types of antibiotics (37)

Table (6) The number of Staph.aureus isolates where resistance for *AMPc* & bla_{CTX} gene

strains	Resistance for antibiotic	Resistance for *AMPc* gene	Resistance for bla_{CTX} gene
1	AM,AX,CIP,KF	+	-
2	AM,AX,GN,COT	+	-
3	AM,AX,CTX,KF,COT	+	-
4	AM,AX,DO,COT	+	-
5	AM,AX,E,CRO,C	-	-
6	AM,AX,E,KF,NIT	+	-
7	AM,AX,CTX,E,GN	+	-
8	AM,AX,E,COT,CIP	-	-
9	AM,AX,C,COT,CRO	-	-
10	AM,AX,E,C,CTX,DO	-	-

+ contain on gene - doesn't contain

Table (7)Show absorption of the most important bands in the infrared spectrum and the Functional group of antibiotic.

Table (7) Absorption of the most important bands in the infrared spectrum to Ampicillin

Band frequency (cm-1)	Band	Mode of Vibration	Functional group
3446.79	-NH	Stretch	Amine (NH2)
2970.38	=CH	Stretch	Aromatic (=CH)
1774.51	C=O	Stretch	Carbonyl of amide
1606.70	C=C	Stretch	Aromatic (C=C)
1496.76	C-N	Stretch	Aliphatic (C-N)

The chemical composition of this antibiotic containing hydroxyl group also appeared (C = O) when absorption (cm-151 1774.). The (C= C) of the aromatic ring appeared (1606.70 cm-1) and as shown in Figure (5, 6, 7). The aim of IR is to diagnosis the chemical bonds (effective groups) in antibiotics and their interaction with nanoparticles where showed that does not a chemical relationship between them, but physicist relationship.

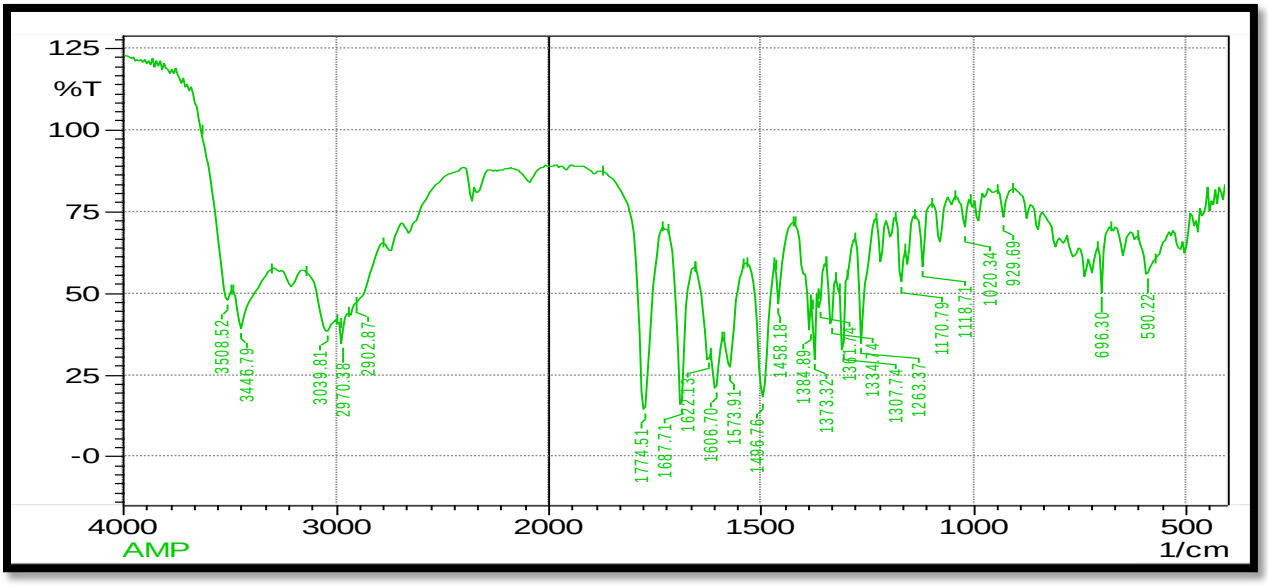

Figure (5) the infrared spectrum for Ampicillin

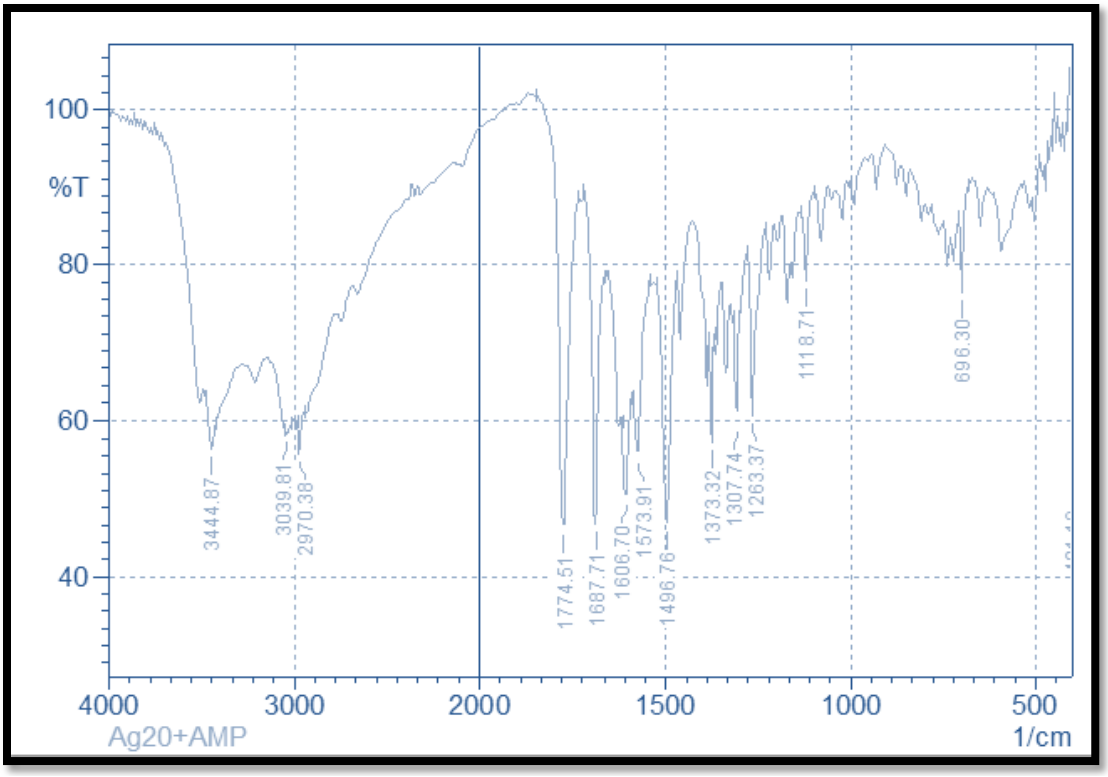

Figure (6) the infrared spectrum for Ampicillin with Ag NPs

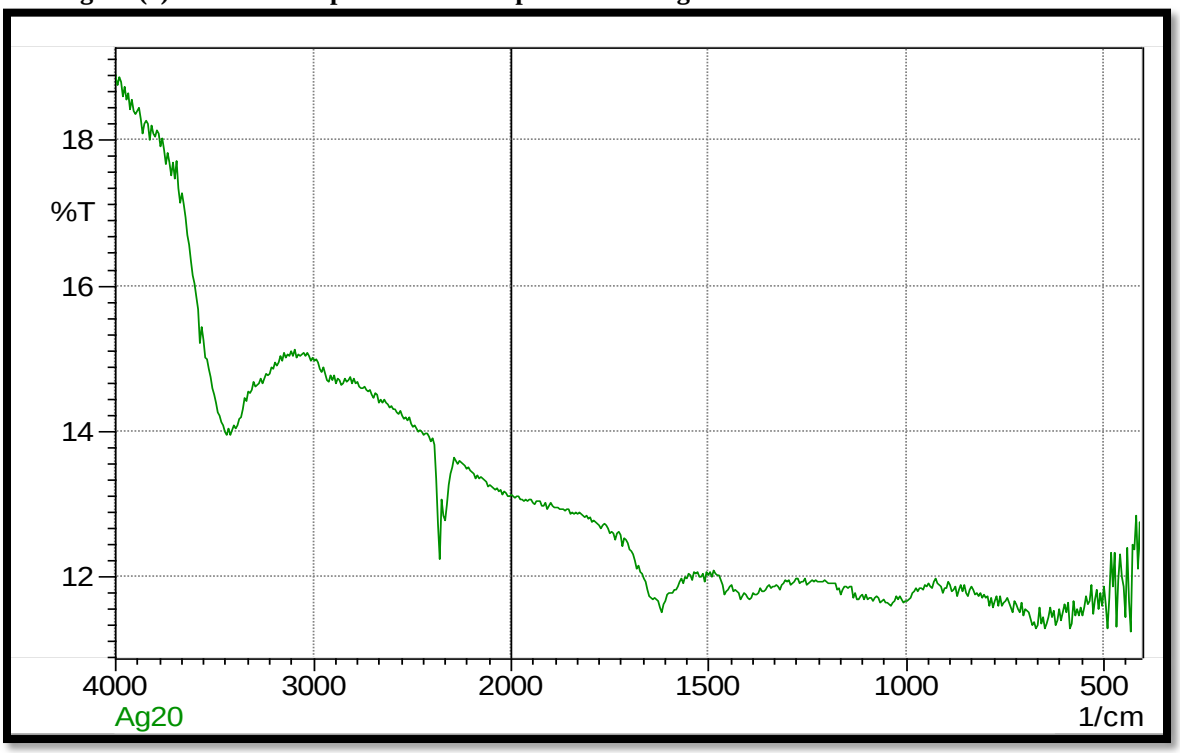

Figure (7) the infrared spectrum for Ag-NP 20nm.

The morphology changes of *Staph.aureus* by Ag &ZnO-NPs solution were investigated with SEM. Figure (8B) the normal spherical shaped of Staph.aureus before treatment obtained from Wikipedia. Figure (8A, C, D) shows SEM image of ZnO NP exposed Staph.aureus. The result showed shape changed during the stress on surface

cell and there are many fragments on the cell surface. Figure (9A, B) .the surface of the cell walls of *S. aureus* was covered with substance resulted from the cell disruption after the Ag-NP treatment indicating the damage of cell surfaces .This is Agree with (38, 39).

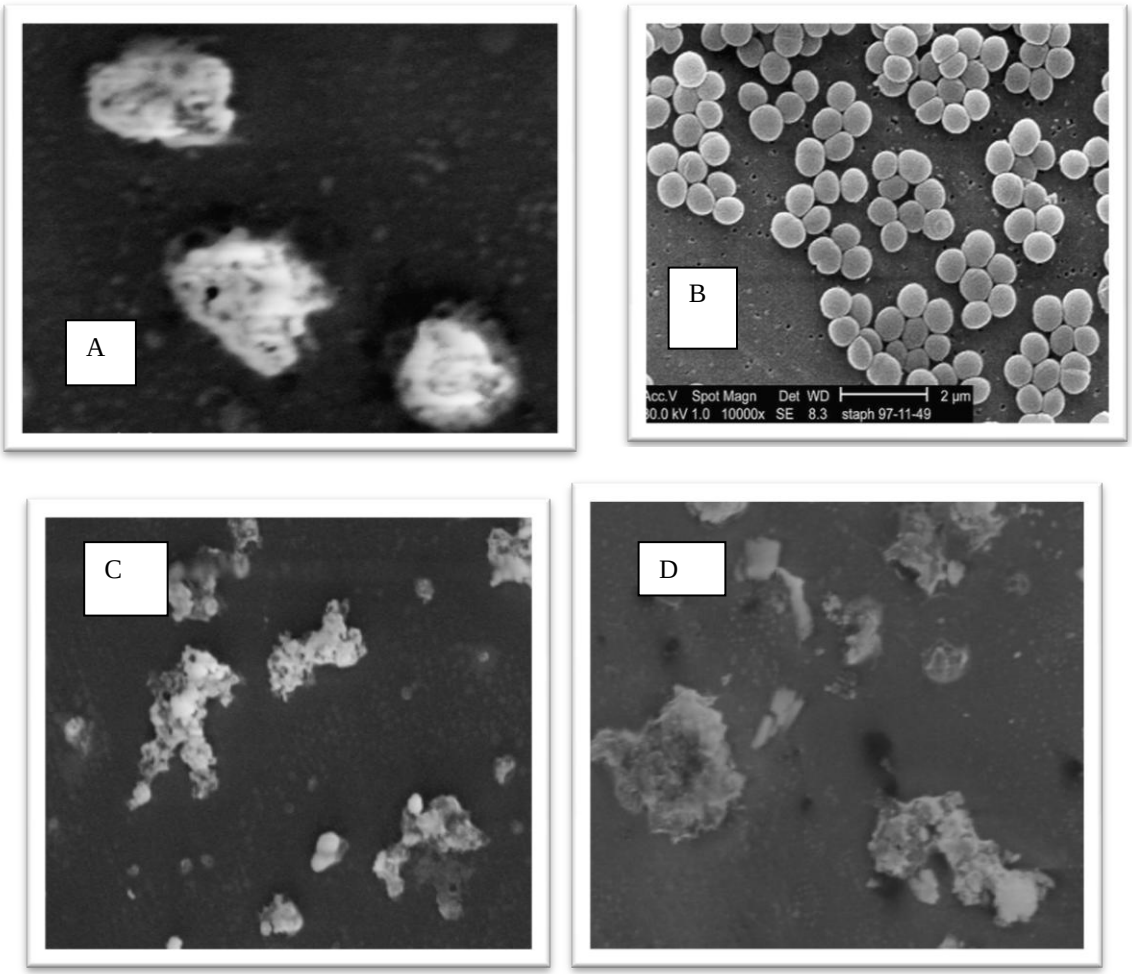

Figure (8) Scanning electron microscopy of *Staph.aureus* with ZnO-NPs (A) 20nm, (B) normal cells (C) 30nm, (D) 50nm.

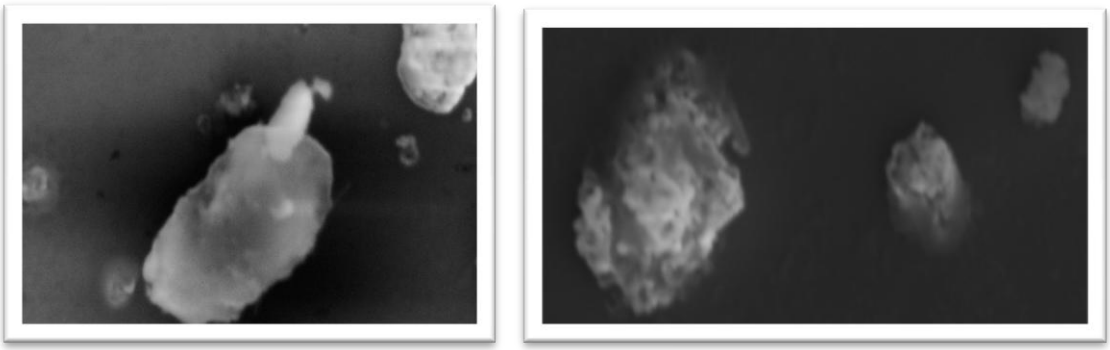

Figure (9) Scanning electron microscopy of *Staph.aureus* with Ag-NPs (lift) 20nm,(right)90nm.

Conclusion

The antimicrobial activity of the nanoparticles showed that the ZnO and AgNPs have great potential to be used as antimicrobial agents against microorganisms.

Resistance to antimicrobial agents by pathogenic bacteria has emerged in recent years and it is a major health problem. There was a synergistic effect between antibiotic and nanoparticles on *Staph.aureus* strains multiple resistance to antibiotics and the AMP_C gene that responsible for resistance didn't found in all resistance strains of *Staph.aureus*.

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