

The Effect of Silver and Titanium Dioxide Nanoparticles on Klebsiella Pneumoniae Isolates Multi Resistant to Antibiotics and Observed by Scanning Electron Microscopy

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Abstract

One hundred fifty six samples collected included: urinary tract infections, wounds, pus, burns and tonsils from patients coming to Rizgary Teaching Hospital and Rozhawa Hospital in Erbil city from March to September 2013, scrubbed and confirmed the diagnosis (33) strain which belongs to *Klebsiella pneumoniae* and by (21.2%) based on cultural characteristics, microscopically features and biochemical tests in addition to the API -20E. These strains sensitivity to 12 types of antibiotics. It gave the species a high resistance against the Ampicillin (AM / 10µg) by 100% and resistant to Amoxicillin (AX / 25µg) by (94%) were less resistant to Cephalothin (KF/30µg), Ceftriaxone (CRO/30µg), Cefotaxime (CTX/30µg) by (36.4, 30.3, 27.3%) 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 titanium dioxide in different sizes (10, 50, 100)nm. The results showed that the MIC for Ag 20nm was between (650 -2600) µg/ml and the MIC for Ag 90nm was between (325 -2600) µg/ml but the MIC for TiO₂ 10, 100nm between (325-2600) µg/ml, MIC of TiO₂ 50nm between (81.25-2600) µg/ml. Synergism effect between the antibiotics and the Nanoparticles when they integrate increased their effect of *Klebsiella pneumoniae*. Morphological changes of bacteria found using scanning electron microscope (SEM) when treating with Nanoparticles. While there a pressure on the bacterial cell surface with losing of bacterial compound.

Keywords: *Klebsiella pneumoniae*; multi-resistant to antibiotics; silver nanoparticles; titanium dioxide and scanning electron microscopy.

Introduction

Nanoparticles were viewed as the fundamental building blocks of nanotechnology [1]. Nanoparticles usually ranging in dimension from 1-100 nanometers (nm). 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 [2]. 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 [3]. 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 [4,5].

Metal oxides NPs such as: ZnO, MgO, TiO₂, SiO₂, CuO and CoO, play a vital role as antimicrobial agents, in other words, these metal nanoparticles can be used as antimicrobial activity because of their effectiveness on resistant strains of microbial pathogens, less toxicity and heat resistance. In addition, they provide mineral elements essential to human cells [6].

The titanium dioxide (TiO₂) Nanoparticle has been used widely to kill Different groups of microorganisms including bacteria, fungi and viruses, because high Stimulus Light [7], the properties of a buffer [8], fixed chemically, low-cost, non-toxic [9]. Because of the photo activity and use in the prevention of sun the number of studies the effects of irradiation on the cells have addressed UVB presence of TiO₂ [10].

The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased in the presence of silver Nanoparticles against both tested bacterial strains [11].

This study was conducted in order to investigate the prevalence of multi-resistance of *Klebsiella pneumoniae* 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 follow-up morphological changes resulting from exposure of bacteria for nanoparticles using a scanning electron microscope.

Materials and Methods

A-Isolation and Identification of Staphylococcus aureus isolates:

One hundred fifty six samples were collected from different human infection (urinary tract infections, wounds, pus, burns and tonsils) from Rizgary and Rozhawa Hospital in Erbil City Since March to September 2013.

The specimens were inoculated on the Nutrient agar and incubated at 37°C for 24 hours. The isolates were examined for their shape, size and color, then transferred and streaked on differential medium for the isolation, purification and identification of *Klebsiella pneumoniae*. All plates were incubated at 37°C 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 [12,13] also API- 20E was used for diagnosis.

B-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 [14]. The antibiotic discs used in this study were Ampicillin (AM/10 µg), Amoxicillin (AX/25 µg), Gentamicin (GM/10 µg), Erythromycin (E/15 µg), Ciprofloxacin (CIP/5 µg), Chloramphenicol (C/30 µg), Cefotaxime (CTX/30 µg), Nitrofurantoin (NIT/300 µg), Co-Trimoxazole (COT/25 µg), Cephalothin (KF/30 µg), Doxycycline (DO/30 µg), Ceftriaxone (CRO/30 µg). Each antibiotic concentration was applied on the surface of Muller -Hinton agar plates and inoculated with *Klebsiella pneumoniae* isolates and incubated at 37°C for 24 h [15].

C-Minimum Inhibitory Concentration (MIC) of Antibiotic and Nanoparticles against K. pneumoniae 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 1024 µg/ml, therefore two fold dilutions were done, the stock solution diluted to (512 µg/ml). All stocks of antibiotic solutions were kept on ice until use.

Commercially synthesized Ag and TiO₂ NPs were purchased from M K Impex Corp., CANADA. The reported “as manufactured” sizes were: AgNPs\20, 90 nm and TiO₂ NPs/ 10, 50, 100 nm. Preparation Stock solution according to the method [16] 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.

D-Inoculums preparation

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

E-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 [17, 18]:-

- 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° 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.

G-Determination of antimicrobial activity and Synergistic effect of silver and titanium dioxide nanoparticles with antibiotics by well-diffusion method

The antibiotics, TiO_2 and Ag NPs were tested for antimicrobial activity by well-diffusion method against *K. pneumoniae* 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 titanium dioxide 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 [19].

H-Scanning electron microscope (SEM)

Sample preparation: It is performed according to [20, 21, 22] as follows:-

1. 100 μl of *K. pneumoniae* suspension which prepared from SubMIC of each different nanoparticle were fixed with 100 μl 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 33(21.2%) *K. pneumoniae* were isolated which based on morphological characteristics, biochemical tests and API - 20E. 15(46 %) isolates were from Urinary tract infection (UTI)

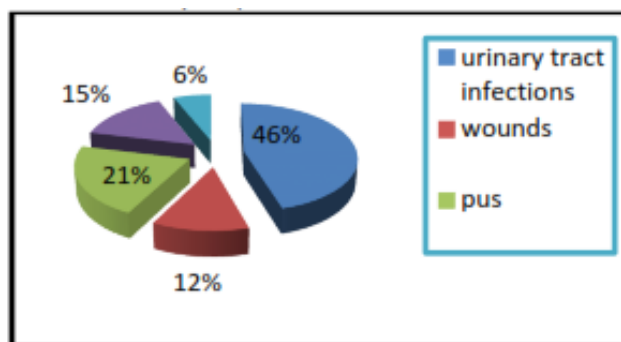


Figure1. Proportions of *Klebsiella pneumoniae* isolated from different sources

K. pneumoniae isolated were identified using traditional morphological and biochemical diagnostic tests according to the [23] and was in agreement as shown in (Table 1). The confirmatory identification of *K. pneumoniae* was performed using API-20E system as shown in figure (2).

TABLE 1. Morphological and biochemical tests for identification of *Klebsiella pneumoniae*

Test	Result	%	Test	Result	%
Gram stain	-	100	Indole	-	100
Catalase	+	100	Methyl red	-	97.6
Oxidase	-	100	Citrate	+	100
Voges proskour	+	100	Urease	+	92.8



Figure (2) API-20E for *Klebsiella pneumoniae*

Figure (3) that there is variation in the resistance isolates under study to the antibiotics used as shown resistance 100% Ampicillin and the resistance 94% of the Amoxicillin, while resistance to Doxycycline and Erythromycin at (81.8, 78.8)% respectively. 54.4% of the Gentamicin, while resistance to Ciprofloxacin 33.3% and to Chloramphenicol 39.4%. They found low resistance to antibiotics cephalosporin and

included Cephalothin, Ceftriaxone, Cefotaxime ratio (36.4, 30.3, 27.3) % respectively. Due to the high effectiveness and wide spectrum against intestinal family members [24]. The isolates were resistant to Nitrofurantoin and Co-Trimoxazole were (45.5, 42.4) % respectively.

Determined minimum inhibitory concentration MIC of antibiotics studied where adopted on Break point described by [25] as the basis for calculating the response. Results in the table (2) showed that MIC values of all *K. pneumoniae* isolates were resistant to Ampicillin, Amoxicillin, Cefotaxime, Cephalothin and Ceftriaxone as values ranged between (32-512) µg/ml. It has been attributed to the production of enzymes and a wide spectrum while the MIC values for Gentamicin and Erythromycin were ranged between (8-256) µg/ml.

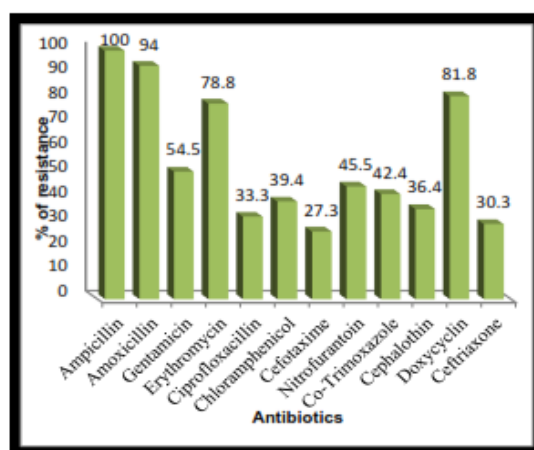


Figure (3): Represents antibiotic sensitivity to *Klebsiella pneumoniae*

The results showed resistant isolates to Co-Trimoxazole where MIC values ranging from (64-512) µg /ml also showed (5) isolates resistance against Chloramphenicol ranging MIC values between (8-128) µg /ml, while all isolates were sensitive to Nitrofurantoin this is precisely what [26] there are generally higher MIC values of various antibiotics and this is due to the prevalence of the use of antibiotics compared to previous years, leading to the emergence of resistant isolates high.

Table 2. MIC value for 12 antimicrobials ($\mu\text{g/ml}$) against *K. pneumoniae* isolates

Isolates Antibiotics	*Break points ($\mu\text{g/ml}$)	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
	MIC ($\mu\text{g/ml}$)										
AM	8<	32	128	128	256	32	64	128	512	64	256
AX	8<	256	128	512	64	256	512	64	128	512	512
GM	4<	64	16	256	8	8	32	64	16	16	32
E	1<	16	32	256	128	16	64	32	16	8	16
CIP	1<	16	8	32	16	4	4	8	16	32	8
C	8<	4	8	128	32	16	1	4	4	8	2
CTX	2<	64	128	256	64	32	128	32	64	128	512
NIT	≤ 64	32	32	4	16	16	32	8	4	1	32
CoT	4<	512	128	512	256	128	512	128	64	256	128
KF	2<	64	512	32	128	256	128	64	32	128	512
DO	2<	8	16	16	32	8	32	512	128	32	64
CRO	2<	256	128	512	64	64	128	32	64	32	512

K: strain, *Standard breakpoints MIC of antibiotic (BSAC, 2012)

Table (3) showed that the MIC for Ag20nm was between (650 -2600) $\mu\text{g/ml}$ and Ag90nm was between (325 -2600) $\mu\text{g/ml}$. silver nanoparticles minutes effect on the cell wall for bacteria, which makes cellular components dispersed and is not as regular affect the DNA, thereby hindering the growth of bacteria [27].

The MIC for (TiO₂-NP) with a size of 10 & 100 nm on isolates of bacteria *K. pneumoniae* had concentrations between (325-2600) $\mu\text{g/ml}$ while the MIC for (TiO₂-NP) a 50nm size was between (81.25-2600) $\mu\text{g/ml}$

The mechanism in which the nanoparticles interaction with the bacterial cells is that the microorganisms carry a negative charge, while the metal oxides carry a positive charge, which creates electromagnetic skirmishes between the bacteria and the surface of nanoparticles, and that the nanoparticles launches ions that react with group (-SH) for proteins transporting materials food that stand out from the bacterial cell membrane there by reducing the permeability of the membrane and thus cell death [28].

Table 3. MIC value for Ag&TiO₂ nanoparticles (µg /ml) against *K. pneumoniae* isolates

Isolates	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
Nano	MIC (µg/ml)									
Ag20	2600	1300	2600	1300	650	1300	650	1300	2600	1300
Ag90	1300	1300	2600	650	325	1300	325	1300	1300	2600
TiO ₂ 10	650	1300	1300	2600	650	325	325	650	1300	650
TiO ₂ 50	1300	1300	325	650	1300	1300	81.25	650	2600	1300
TiO ₂ 100	650	325	2600	1300	650	2600	1300	650	1300	1300

Table (4) shows the effect of the relationship between antibiotics and nanoparticles on *K. pneumoniae*, where the results showed that there was a marked increase in Diameter rate inhibition zone when a combination of antibiotics and nanoparticles called case Synergism. Increasing the rate of inhibition zone when mixing (GM, C, CIP, CTX, NF, DO) with silver nanoparticles and various sizes.

Table 4. Synergistic effect of nanoparticles with antibiotics on *K. pneumoniae* isolate

Antibiotics	Effect of antimicrobial agents alone	Effect of Antibiotic & Nanoparticles(mm)					Effect of Nanoparticles(mm)				
		Anti+ Ag20	Anti+ Ag90	Anti+ Tio ₂ 10	Anti+ Tio ₂ 50	Anti+ Tio ₂ 100	Ag 20	Ag 90	Tio ₂ 10	Tio ₂ 50	Tio ₂ 100
Am	0	0	0	0	0	0	0	0	0	0	0
AX	0	0	0	0	0	0					
GM	20	23	22	18	17	18					
E	0	0	0	0	0	0					
CIP	18	19	20	18	19	17					
C	21	22	24	22	18	17					
CTX	18	20	22	16	15	17					
NF	17	18	19	15	20	15					
COT	0	0	0	0	0	0					
KF	0	0	0	0	0	0					
DO	19	21	20	22	22	20					
CRO	0	0	0	0	0	0					

Morphological variations gram negative bacteria clarified figure (4- A, B) where he showed *K. pneumoniae* cells when treated with silver nanoparticles sizes (20,90nm) change in the bacterial cell surface and this was confirmed by [29] through studying the mechanism of the effect of silver nanoparticles on *K. pneumoniae* and also Confirmed the researcher [30,31] that the morphological changes on the cell surface occurs due to increased permeability output when exposed to silver nanoparticles, showing images (4- C, D,E) for damage to bacterial cell by pressure on the surface cell when exposed bacteria dioxide titanium sizes(10,50,100nm)

The use of nanoparticles as drug carriers may reduce the toxicity of the incorporated drug but it is sometimes difficult to distinguish the toxicity of the drug from that of the nanoparticle. Toxicity of gold nanoparticles, for instance, has been shown at high concentrations. In addition, nanoparticles trapped in the liver can affect the function of this organ. [32]

Nanoparticles have the potential to cross the blood brain barrier, which makes them extremely useful as a way to deliver drugs directly to the brain

Conclusion

The antimicrobial activity of the nanoparticles showed that the AgNPs and TiO₂ NPs 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 *K. pneumoniae* strains multiple resistance to antibiotics

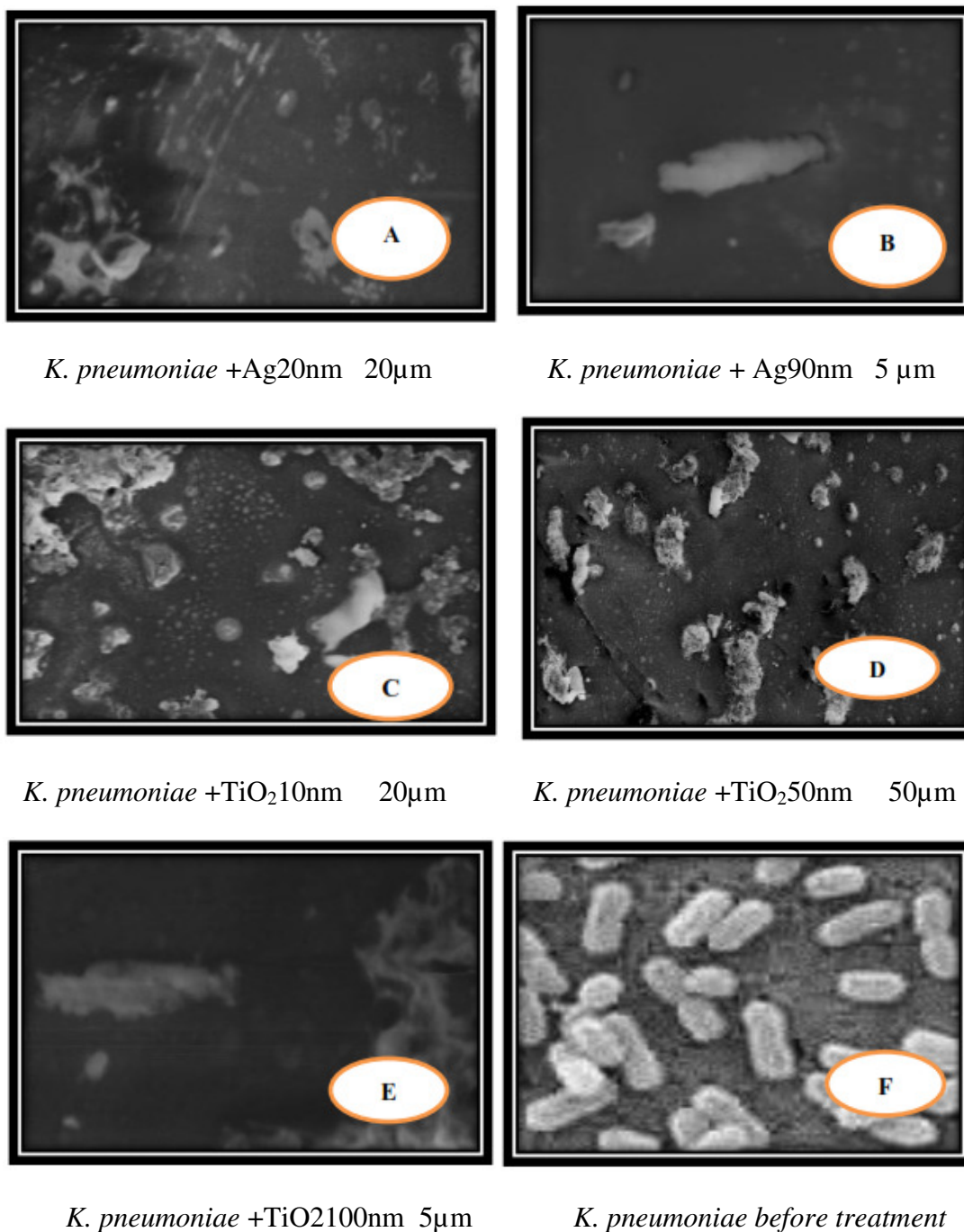


Figure 4. Morphological changes of *K. pneumoniae* when observed by Scanning electron microscopy that (A) when the magnification power of 42066 X, (B) at 17782X, (C) at 16033X, (D) at 3671X, (E) at 21652X.

Reference

- [1] A. Mansoori, T.F. George, G. Zhang and L. Assoufid ,“ Molecular Building Blocks for Nanotechnology,” *Springer*, New York, 2007.
- [2] K .Okuyama and W. Lenggoro, “Nanoparticle preparation and its application – A nanotechnology particle project in Japan,” Computer Society. (ICMENS’04). 2004.
- [3] , F.M. Gutierrez, , P.L. Olive, A. Banuelos, E. Orrantia, N. Nino, E.M. Sanchez, F. Ruiz, H. Bach, Y.A. Gay, “Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles,” *Nanomedicine*, 2010, 6:681-688.
- [4] K.H. Cho, J.E. Park, T. Osaka, S.G. Park, “The study of antimicrobial activity and preservative effects of nanosilver ingredient,” *ElectrochimActa*, 2005, 51:956–960.
- [5] C.N. Lok, C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H. Sun, P.K. Tam, J.F. Chiu, C.M. Chen, “ Proteomic analysis of the mode of antibacterial action of silver nanoparticles,” *J. Proteome Res.*, 2006 , 5:916–924.
- [6] Z.E. Karvani and P. Chehraz, “Antibacterial activity of ZnO nanoparticles on gram-positive and gram-negative bacteria,” *African Journal of Microbiology Research*, 2011, 5(12): 1368-1373.
- [7] N.S. Allen, M. Edge, J. Verran, J. Stratton, J. Maltby and C. Bygott, “Photocatalytic Titania based surfaces: Environmental benefits,” *Polym. Degrad. Stab*, 2008, 93: 1632–1646.
- [8] X. Chen and S .S. Mao, “Titanium Dioxide Nanomaterials: Synthesis, Properties, Modifications and Applications,” *Chem. Rev.*, 2007, 107: 2891-2959.
- [9] T. Sugimoto, X .Zhou and A. Muramatsu, “Synthesis of uniform anatase TiO₂ Nanoparticles by gel-sol method: 3. Formation process and size control,” *Journal of Colloid and Interface Science*, 2003, 259(1): 43–52.
- [10] T. Scown, “Uptake and Effects of Nanoparticles in Fish,” Ph. D. dissertation submitted to the University of Exeter, Biological Sciences, and September. 2009.
- [11] R. Shahverdi, A. Fakhimi, H. R. Shahverdi, S. Minaian, “Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*,” *Nanomedicine*, 2007, 3, 168-171.
- [12] J.P. Harley and L.M. Prescott, “Laboratory Exercises in Microbiology,” 5th.ed. The McGraw-Hill Companies, Inc. New York. 2002.

- [13] W. Winn, S. Allen, W. Janda, et al, "Koneman's Color Atlas and Textbook of Diagnostic Microbiology," 6th Ed., Lippincott Williams and Wilkins Philadelphia, 2006, pp. 218,648, 947-982, 1446-1457.
- [14] CLSI, (Clinical & Laboratory Standards institute), "Performance standard for antimicrobial susceptibility testing," Twenty -first informational supplement. M100-S21, 2013, 33 (1):101-106.
- [15] S.S. Tankhiwale, S.V. Jalgaonkar, S. Ahamad and U. Hassani, "Evaluation of extended spectrum beta lactamase in urinary isolates," Indian J Med Res, 2004, 120:553–556.
- [16] M.A. Ansari, M.K. Haris, A.K. Aijaz, S. Asfia, A. Ameer, "Synthesis and characterization of the antibacterial potential of ZnO nanoparticles against extended-spectrum β -lactamase-producing *E. coli* and *K. pneumoniae* isolated from a tertiary care hospital of North India," Appl. Microbiol Biotech, 2009, 10:3733–3736.
- [17] Amsterdam, "Susceptibility testing of antimicrobials in liquid media. In: Loman, V., ed. Antibiotics in laboratory medicine," 4th ed. Williams and Wilkins, Baltimore, MD. 1996, p.52-111.
- [18] R. Saginur, M.S. Denis, W. Ferris, S.D. Aaron, F. Chan, C. Lee and K. Ramotar, "Multiple combination bactericidal testing of *Staphylococcal* Biofilms from implant-associated infections," *Antimicrobial Agents Chemother*, 2006, 50(1): 55-61.
- [19] N.S. Egorove, "Antibiotic asientific approach Mir publishers," Moscow, 1985.
- [20] Y. Chao and T. Zhang, "Optimization of Fixation Methods for Observation of Bacterial Cell Morphology and Surface Ultra-structures by Atomic Force Microscopy," J. Appl. Microbiol. Biotechnology, 2011, 92:381-392.
- [21] S. AL-Bahry, N. Sivakumar and M .AL-Khambashi, "Effect of Nalidixic acid on the Morphology and protein Expression of *Pseudomonas aeruginosa*," Asian pacific J. of typical Medicine, 2012,p: 265-269.
- [22] R.K.F. Al-Taesh and W.M. Ra'oof , "Scanning electron microscope of disinfections exposed *Pseudomonas aeruginosa* isolated from different types of infections," *International Journal of Technical Research and Applications*, 2014, 2(3):75-79.
- [23] Goldman and H. G. Lorrence, "Practical Handbook of Microbiology," 2nd ed. Taylor and Francis Group.USA, 2009.

- [24] E.J. Baron, P.R. Murray, F.C. Tenver and R.H. Yolk, "Manual of Clinical Microbiology," 6th Ed .American Society for Microbiology .U.S.A. 1999.
- [25] BSAC, "Report of the working party on antibiotic sensitivity testing of the British Society of Antimicrobial Chemotherapy," Version 11.1, 2012, PP: 1-91.
- [26] P. Singh and R. Balaji, "Synergistic effect of silver nanoparticles with the cephalixin antibiotic against the test strains," *Bioresearch Bulletin*, 2012, 4: 171-179.
- [27] S.M. Ouda, "Some Nanoparticles effects on *Proteus sp.* and *Klebsiella sp.* isolated from water," *American Journal of Infectious Diseases and Microbiology*, 2014, 2(1):4-10.
- [28] Zhang and G. Chen, "Potent Antibacterial Activities of Ag/TiO₂ Nan composite Powders Synthesized by a One-Pot Sol-Gel Method," *Environ Sci. Technol.*, 2009, 43(8): 2905-2910.
- [29] W. Li, X. Xie, Q. Shi, H. Zeng, Y. OU-Yang and Y. Chen, "Antibacterial activity and mechanism of silver nanoparticles on *Escherichia coli*," *Appl. Microbiol. Biotechnol*, 2010, 85:1115–1122.
- [30] Q. L J. Feng, G. Q. Wu, F. Z. Chen, T. N. Cui and J. O. Kim, "A Mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*," *J. Biomed. Mater. Res.*, 2000, 52:662-668.
- [31] S. Pal, Y., Tak and J. Song, "Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*," *Applied and environmental microbiology*, 2007, 73(6), 1712-1720.
- [32] R. J. White, V. L. Budarin, J. W Moir and J. H. Clark, "A sweet Killer: mesoporous polysaccharide confined silver Nanoparticles for Antibacterial applications," *Int .J. Mol. Sci.*, 2011, 12:5782-5796.