International Journal of Applied Engineering Research ISSN 0973-4562 Volume 12, Number 24 (2017) pp. 14828-14832


# Antimicrobial effect of Sliver Nanoparticles Synthesized by Chemical Reduction on some Bacteria and Fungi

**Dr. Nebras N. Al-Dabbagh[1], Fattima Al-Zahraa G.Gassim[2] and Nada Khazal Kadhim Hindi [3,*]**

*[1]Ph.D. Medical Microbiology, Microbiology Department, College of Dentistry, University of Babylon, Babylon Province, Iraq.*

*[2]MSc. Department of pharmacies, College of Pharmacy, University of Babylon, Iraq.*
*[3]Assistant Professor, .Ph.D. in Microbiology, Basic and Medical science Department, College of Nursing, University of Babylon, Babylon Province, Iraq.*
*(*corresponding author)*

**Abstract**

**Background;** Nanotechnology involves the tailoring of materials at atomic level to attain unique properties, which can be suitably manipulated for the. Most of the natural processes also take place in the nanometer scale regime.

**Aim**; The purpose from this experiment was to describe antimicrobial effects of sliver nanoparticles on some bacteria and fungi.

**Method**; The morphology and size of particles of nanoparticles were determined by scanning electron microscopy (SEM) and Visible absorption spectrophotometer.

**Results;** The antimicrobial effect was studied using the well agar diffusion and broth dilution techniques. The MIC of sliver nanoparticles were 30 ppm for *Candida albicans*, *Candida tropicalis* and *Candida Krusei*. Compared to other strains (*E. coli, Pseudomonas aureginosa*, *S. aureus*) 100 ppm. The antimicrobial activity was observed against all tested microorganisms at different concentration of nano sliver. The Ag NPs causes growth delay for treated isolated with different concentration used in the experiment (increased concentration, increased growth inhibition).

**Conclusion**; The study revealed that sliver nanoparticles can be further developed as an antimicrobial agent, hence high activity against microorganisms.

**Keyword;** Antimicrobial effect, Sliver Nanoparticles, bacteria and fungi

## INTRODUCTION

Nanotechnology involves the tailoring of materials at atomic level to attain unique properties, which can be suitably manipulated for the [1-3] Most of the natural processes also take place in the nanometer scale regime. Therefore, a confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine [4-5]. The Ag-NPs have distinctive physical and chemical properties, such as, chemical stability, high thermal and electrical conductivity, nonlinear optical behavior, surface - enhanced Raman scattering and catalytic activity [6 -7]. These properties made this nanoparticls to the top of the priority list, to be used in inks, in electronics, and of course biology[8-12]. For a long time silver ion has been used as antimicrobial agent since time immemorial in the form of metallic sliver nitrate, sliver sulfadiazine for the treatment of wounds , burns due to its strong inhibiting effect on bacteria, Viruses, fungi, protozoa[13-22] . Due to their proven antimicrobial properties, Ag-NPs are widely used in the daily used commercial products, such as cosmetics, detergent, plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent [23- 25] **Aim**; The purpose from this experiment was to describe antimicrobial effects of sliver nanoparticles on some bacteria and fungi.

## MATERIALS

### Microorganisms

In this study three isolates of bacteria; *E. coli, S. aureus* (MRSA), *Pseudomonas aeruginosa* and three fungal strains, *Candida albicans*, *Candida tropicalis, and Candida krusei*. The bacteria were grown in the Nutrient broth at 37$^{o}$C , using Nutrient agar slants for sub culturing and Sabourauds broth at 37 $^{o}$C for fungi using Sabourauds agar slants for subculturing, and preserved all the isolates in refrigerator at 4$^{o}$C .

### Synthesis of sliver nanoparticles:

The AgNPs prepared from a volume of 100 ml sliver nitrate solution with 1 x $10^{-4}$ mol / l was mixed with 300 ml of 2 x $10^{-2}$ mol /l sodium borohydride both solution was immersed in ice bath. The reaction protected from light. After the addition of sliver nitrate solution was finished, the mixture was than vigorously stirred during 15min .

### Characterization of sliver nanoparticles

Sliver nanoparticles were characterized spectrophotometrically using vis spectroscopy analyses .Scanning electron microscopy (SEM) has been employed to

characterize the shape and morphologies of formed synthesized of AgNPs.[26]

**Antimicrobial activity of sliver nanoparticles:** ***Determination of Minimum inhibitory concentration and Minimum Bactericidal / fungicidal concentration.***

Inoculation suspensions of the *Candida albicans, Candida tropicalis, Candida krusei* were prepared by picking colonies from 24 h Sabraudous agar at $37C^0$ . Also *S. aureus, E. coli, P. aeruginosa* were prepared by picking colonies from nutrient agar (24h) and using sterile saline (0.85%) v/v NaCl to determine turbidity equivalent to 0.5 McFarland standard ($1\times 10^8$) colony forming units /ml.

Broth dilution method was followed for measurement of MIC values . Different concentration of nanoparticles sliver solution(10,20 ,30,40,50,100, 150, 250, 300, 400 ppm ) were added to LB broth medium , the test tubes were incubated at $37C^0$ . The MIC values ( Bacteriostatic / Fungistatic ) were estimated the lowest concentration in the test tube that showed no turbidity ( growth of isolate) after incubation . The minimum bactericidal / fungicidal concentration (MBC / MFC) was evaluated through sub culturing from each test tube showing no apparent growth on the agar after further 24hr incubation.

***Antimicrobial susceptibility testing using Agar well diffusion assay NCCL[27]***

The antibacterial susceptibility of prepared AgNPs against fungi isolates and bacteria isolates were evaluated by Agar well diffusion method . Loop full of freshly – grown bacterial / fungal isolates were inoculated in LB(Luria broth ) incubated at 37 $C^0$ for 24hr. .The bacterial suspensions were diluted with normal saline. A just the tubidity and compare with standard tube (McFarland number 0.5)to yield a uniform suspension containing $1.5\times 10^8$ CFU / ml and spread the suspension by cotton swab , streaking on LB agar Media were cut into four wells (5 mm diameter) by cork borer and add 30µ of suspension (different concentrations) (the plates were performed **i**n triplicates). All plates of the tested organisms was then allowed to incubate at 37 $C^0$ for overnight. Reading the result by the measuring the distance / inhibition zones around the wells .

## RESULTS AND DISCUSSION

### UV- visible analysis

The absorption spectrum was measured immediately after preparation . UV – visible spectroscopy is one of the most widely and sensitive techniques for structural characterization of sliver nanoparticles synthesis, Figure (1) shows the maximum absorption peak was observed at about 413nm which is the characteristic absorption peak for Ag nanoparticles.

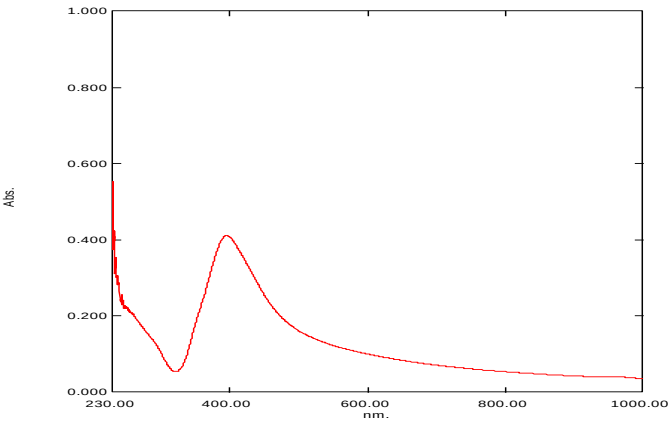


**Figure 1:** Uv – Vis spectra- The maximum absorbance was at 413 nm

### SEM analysis :

SEM was used for observing the morphology of synthesized AgNPs. Fig.(2) shows the SEM images of AgNPs. As , It can been seen that the inner diameter of the AgNPs is ranging beteewn (65-72 )nm.

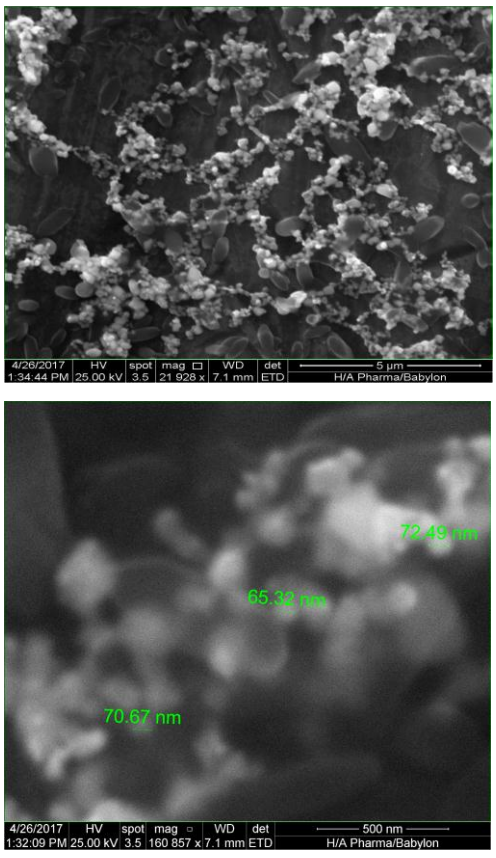


**Figure 2:** SEM image of Ag nanopartcals.

**Antimicrobial activity of sliver nanoparticles**

Table (1) showed the antimicrobial properties of Ag- NPs were studied against isolates**,** *S. aureus* ( MRSA), *E.coli* , *P.aeruginosa Candida albicans, Candida tropicalis* , *Candida krusei.* The Minimum inhibitory concentration of Ag –NPs against *Candida albicans, Candida tropicalis* , *Candida krusei* were found 30 ppm , 30ppm , 30 ppm , respectively. The MICs of AgNPs were 100 ppm for other bacterial test strains, including *S.aureus (*MRSA) , *E.coli* , *Pseudomonas aeroginosa* .While the Minimum Bacterial / Fungicidal Count of Ag –NPs against *Candida sp.* were found 300 ppm **.** It was observed from MIC values obtained in broth dilution method that antimicrobial efficiency of Nanosliver solution was higher against yeasts compared to bacteria . Fig (3) showed inhibition zones increases with increasing concentration of sliver. Similar results have been reported on some bacteria and fungi [28-30].Some authors demonstrate different values in MIC results for determining antimicrobial activity of sliver nanoparticles against bacteria and fungi due to general physiological differences in the cell wall membrane of microorganisms[31]. Indeed to that other scientific studies referred to Ag NP activity depend on the their concentration , size and shape [32-35]

Also antimicrobial activity of sliver ions bind to the protein and nucleic acid negatively charged ,causing structural changes and deformations in the (wall, membranes , nucleic acids) . Sliver ions interact with a number of electron donor functional groups such as hydroxyls, imidazoles, thiols, phosphates and indoles. Also AgNPs induce and release of reactive oxygen species (ROS), forming free radicals . AgNPs can enter the bacteria or fungi and damage of its cellular structures, inhibition of protein synthesis as a result of ribosomes denaturation, as well as translation and transcription will be blocked by the binding with genetic material of the bacteria[36-39] .

**Table 1:** Determination of MIC and MBC for sliver nanoparticles for isolates

| Isolate | MIC Conc.(ppm) | MBC Conc.(ppm) |
|---|---|---|
| *C. albicans* | 30 | 300 |
| *C. tropicalis* | 30 | 300 |
| *Candida krusei* | 30 | 300 |
| *S. aureus* | 100 | 300 |
| *E.coli* | 100 | 300 |
| *P. auroginosa* | 100 | 300 |

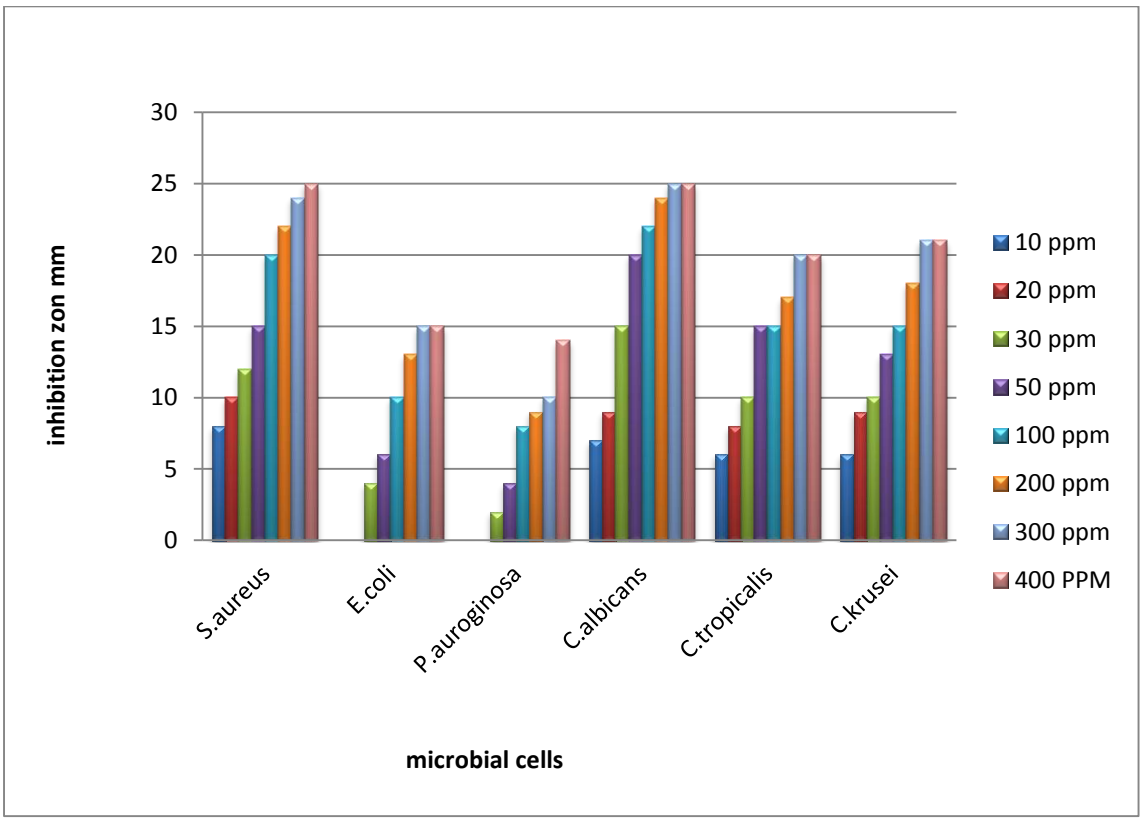


**Figure 3:** Antimicrobial activity of Ag- NPs against bacteria and fungi

## CONCLUSION

In this study, simple and effective method of synthesis of AgNPs solution using a chemical reduction method , was tested against six isolates .Different degrees of sensitivity were shown among isolates. The study conclude that Ag-NPs have potent antimicrobial activities against tested isolated . therefore , it was proposed as an alternative drug for antimicrobial activity and can inhibit the Gram positive , gram negative growth and yeast growth .

## REFERENCES

[1] Gleiter H. Nanostructured materials, basic concepts and Microstructure . Acta Mater, 2000, 48 1-12 .

[2] Chaloupka K.,MalamY.,SeifalianAM .Nano sliver as a new generation of nano product in biomedical applications. Trends Biotechnol., 2010 , 11: 580- 88.

[3] Mihail C Roco . The long View of Nanotechnology Development :National Nanotechnology Initiative at 10 years. In :Mihail C Roco et al .(Eds.) Nanotechnology Research Directions for Societal Needs in 2020 Science Policy Reports,Springer , 2011 , 1. 1- 28.

[4] Brett DW. A discussion of sliver as an antimicrobial agent : Alleviating the confusion .Ostomy wound Manage , 2006; 52:34-41.

[5] Marambio-Jones , C,Hoek EMV. A. review of the antibacterial effects of sliver nanomateriales and potential implications for human health and the environment .J.Nanopart. Res. 2010;12(5): 1531-1551.

[6] Gupta AK,Gupta M.Synthesis and surface engineering of iron oxide nanoparticles for biomedical application Biomaterials .2005; 26: 3995 – 4021.

[7] Jamieson T,Bakhshi R,Petrova CD,Pocock R,Imani MSeifalian AM. Biological applications of quantum dots. Biomaterials , 2007; 28 : 4717- 4732.

[8] Roco MC . Nanotechnology convergence with modern biology and medicine . Curr Opin Biotechnol , 2003;14 (3): 337- 346.

[9] Kyriacou SV Brownlow Wj ,Xu XH. Using nanoparticle optics assay for direct observation of the function of antimicrobial agents in single live bacterial cells .Biochemistry, 2004 ; 43(1): 140 – 147.

[10] Rudolf G, Groppel P.Volker M, Muller N. Application of Nanoparticles in Polymers for Electronics Engineering . Particle & systems Characterization , 2002 ;19(5): 293 – 299.

[11] Lui, V. K. M. Poon, I. Lung, and A. Burd. Antimicrobial activities of silver dressings: an in vitro comparison," Journal of Medical Microbiology , 2006 ; 55,( 1): 59– 63

[12] GogosA, Knaure K, Bucheli TD . Nanomateriales in plant protection and Fertilization . Current State , Foreseen Applications and Research Priorities. JAgric Food Chem , 2012 ; 60 (39): 9781- 9792.

[13] Dowsett C . The use of sliver – based dressings in wound care . Nurs Stand , 2004;19 (7) : 56- 60.

[14] Melaiye A, Youngs wj . Sliver and its application as an antimicrobial agent. Expert Opin Ther. Pat, 2005; 15 : 25 – 30.

[15] Lansdown A. Sliver in health care : antimicrobial effects and softy in use .Curr Probl Dermatol, 2006 ; 33: 17- 34.

[16] Choi O,Hu Z . Size dependent and reactive oxygen species related nanosliver toxicity to nitrifying bacteria .Environ Sci Technol , 2008;42 (12): 4583-4588.

[17] Jung WK , Koo HC,Kim KW,ShinS,KimSH, ParkYH . Antibacterial and mechanism of action of the sliver ion in Staphylococcus aureus and Escharichia coli .Appl.Environ. Microbiol, 2008 74:2171- 2178.

[18] Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee D G. Antifungal effect of sliver of sliver nanoparticles on dermatophytes . J.Microbiol Biotechnol , 2008;18,1482-1484.

[19] Nanda A,Saravanan M. Biosynthesis of sliver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. Nanomedicine , 2009 ; 5:452- 456.

[20] Pinto DB,Shukla S,Perkas N, GedankenA,Sarid R. Inhibition of Herpes Simplex Virus Type 1Infection by Sliver Nanoparticles Capped with Mercaptoethane Sulfonate Bioconjugate Chem . 20 : 1497- 1502.

[21] Kim K J, Sung W SJ, Suh Bk, Moon Sk, Choi JS, Kim JG& LeeD G. Antifungal activity and mode of action of sliver nano-particles on *Candida albicans* . Biometals , 2009 , 22(2) 235.

[22] Lara HH,Ayala – Nunez NV,Turrent LI,Padilla CR. Mode of antiviral action of sliver nanoparticles against HIV .Journal of Nanobiotechnology , 2010;8: 1-9.

[23] Russell AD, Hugo WB . Antimicrobial activity and action of sliver . Prog Med Chem , 1993; 31: 351- 70 .

[24] Stickler Dj . Biomateriales to prevent nosocomial infections: Is sliver the gold standard ? Curr Opin Infect Dis , 2000;13: 389 -39.

[25] Baker C,Pradhan A PakstisL,Pochan DJ,SI. Synthesis and antibacterial properties of sliver nanoparticles.J.Nanosci. Nanotechmol, 2005;51: 956- 960.

[26] Kim JS, KukE , YuKN, Kim JH, Park SJ, Lee HJ et al. Antimicrobial effects of sliver nanoparticles .Nanomedicine . 2007);3(1): 2346- 53 .

[27] Forbes BA, Sahm DF and Weissfeld AS,Bailey and Scotts·. Diagnostic microbiology. 2007 12th.ed. Elsevier China.

[28] Sondi I, Salopek Sondi B. Sliver nanoparticles as antimicrobial Agent : Acase study on *E.coli* as a model for Gram – negative Bacteria . J Colloid Interface Sci . 2004; 275 , 177- 182.

[29] Panacek ,M. Kilianova , R.Prucek, Husickova , R et al . Preparation and in vitro Bacterial and Fungicidal Efficiency of Nanosliver / Methylcellulose Hydrogel. Inter.J. Chem. Mole. Nucl. Mate. Meta. Eng. 2014 ;8.6..

[30] Nebras N. AL- Dabagh &Fatema . AL- Zahraa . Effect of Sliver nanoparticles solution on *Candida glabrata* isolated from skin infection. AJBAS . 2016; 10(15), 134-139.

[31] Jung WK , Koo HC,Kim KW,ShinS,KimSH, ParkYH. Antibacterial and mechanism of action of the sliver ion in *Staphyllococcus aureus* and *Escharichia coli* .Appl.Environ. Microbiol, 2008 ; 74:2171- 2178.

[32] Periasamy S, Joos HS, Duong AC, TanVY, Chatterjee SS, Cheung G Y, Otto M. How *Staphylococcus aureus* biofilms develop their characteristic structure .Proc.Natl Acad.Sci.USA. ,2012;109, 128- 1286.

[33] Chernousova Epple M. Sliver as antibacterial agent:Ion, nanoparticle, and metal .Angew. Chem.Int.Ed.Engl. ,2013;52 :1636 – 1653.

[34] Taraszkiewicz A, Fila G, Grinholc M, Nakonieczna, J. Innovative strategies to overcome bioresistance.Biomed.Res.Int., 2013;150653.

[35] Pal S,Tak YK , Song JM. Does the antibacterial activity of sliver nanoparticles depend on the shape of the nanoparticle? A study of the Gram – negative bacterium *Escherichia coli*. Appl Environ. Microbio, 2007 ;73(6):1712 – 20 .

[36] Rai , M.,Yadav,A.,Gade A . Sliver nanoparticles as a new generation of antimicrobials Biotechnol.Adv. 2001 ; 27 :76-83 .

[37] Morones JR, Elechiguerra JL, CamachoA, Holt K, Kouri JB Japia J,. The YacamanMJ. Bactericidal effect of sliver nanoparticles. Nanotechnology,2005;16 10: 2346 – 2353.

[38] Kim JS, KukE,YuKN, Kim JH,ParkSJ,LeeHJ et al. Antimicrobial effects of sliver nanoparticles. Nanomedicine .2007; 3(1): 2346- 53.

[39] Kim KJ, Sung W S J, SuhB k, Moon Sk, Choi JSj, Kim JG. & Lee D G. Antifungal activity and mode of action of slive nano-particles on *Candida albicans*. Biometals , 2009;22,2:235.