Azhar Int J Pharm Med Sci 2021; 1 (1):22-31

(Research article)

Open Access

Print ISSN: 2735-4598

Online ISSN: 2735-4601

Enhanced antimicrobial activity of the combination of silver nanoparticles and different β Lactam antibiotics against methicillin resistant *Staphylococcus aureus* isolates

Fatema BD. Abdel-Wahab[1], Nagwan G. El Menofy[1]*, Ahmed I. El-Batal[2], Farag M. Mosallam[2], Abeer K. Abdulall[1]

[1] Microbiology and Immunology Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt

[2] Drug Microbiology Lab., Drug Radiation Research Dep., National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt

* Correspondence: nagwan.elmenofy@azhar.edu.eg

Article history: Received 14-12- 2020 **Revised** 03-01-2021 **Accepted** 06-01-2021

Abstract: Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a major problem worldwide. Strong synergistic action of antibiotics combined with silver nanoparticles (AgNPs) presents a potential solution for this problem. AgNPs was prepared by Gamma- irradiation method. Eighty *staphylococcus spp*. were obtained out of 45 different clinical specimens; 21(46.6 %) isolates were identified as MRSA and chosen to test their susceptibility to plain AgNPs, β-lactam antibiotics and mixed solution of AgNPs /antibiotics by disc diffusion method and broth microdilution assay for detection of minimum inhibitory concentration (MIC) as well as minimum bactericidal concentrations. All MRSA isolates were full resistant to amoxicillin, ceftazidime, cefotaxime, ceftriaxone, cefepime, while 3(14.2%) were susceptible to meropenem and imipenem with MIC ≤ 20µg/ml. Plain AgNPs showed potential anti-MRSA activity, the range of inhibition zones of AgNPs ranged from 7.5 to 12 mm. Broth microdilution assay revealed that 8(38.1%) and 5(23.8%) were inhibited at concentration of 31.25 and 7.81µg/ml of AgNPs, respectively. Disc diffusion assay showed the enhancement of activity of amoxicillin/AgNPs, imipenem/AgNPs and meropenem/AgNPs combinations, the fold increase areas ranging between 33.3 to 100, 0 to 166.6 and 0 to 166.6 respectively. Additionally, MIC of amoxicillin in amoxicillin/AgNPs mixture was decreased from more than 5120 µg/ml to reach 1.95 µg/ml in 28.5% of MRSA and 0.97 µg/mL in 19 % of MRSA isolates. The enhanced activity of antibiotics, especially amoxicillin, combined with AgNPs was very high against the resistant isolates, so utilizing combination therapy is one the proposed approaches to treat MRSA bacterial infections.

Keywords: *Staphylococcus aureus,* Silver nanoparticles, Gamma Irradiation, β-lactams, Broth microdilution

1. INTRODUCTION

An ever-increasing of bacterial resistance to the effects of current antibiotics is one of the most significant health care issues [1,2]. High burden of multidrug-resistant *Staphylococcus aureus* isolates specifically methicillin-resistant *S. aureus* (MRSA) constitutes a problem for patients with compromised immunity or with exposed open access as well to healthcare members. The spectrum of MRSA infection ranged from mild superficial skin infections to severe diseases as bronchopneumonia [3].

In the present time, nanoscale materials have arose as innovative antimicrobial agents owing to their unique physical and chemical propertiesin addition to high surface area to volume ratio [4]. Among the various types of metallic nanoparticles (NP), silver NPs (AgNPs) have proven to be the most effective against highly resistant bacterial isolates [5-9] as they, attack a broad range of target sites and metabolic processes in the organisms [10-13]. Moreover, AgNPs possess intense surface chemistry and stability, proper size (250 times smaller than a bacterium) and able to maintain their shape and size in solution. [6] Gamma-irradiation synthesis of metallic nanoparticles considred as one of the most promising methods for AgNPs preparation as it is more convenient and clean. The radiochemical process produce Ag^+ ions at the ambient temperature without producing toxic by-products of the reductant or using excessive reducing agents [6]. Beta-lactam antibiotics are the principal group of antibiotics have high antibacterial activity and broadly used in clinical applications [14]. Wide use of β-lactam antibiotics has created resistance complications that often mediated by lactamases production leading to

Cite this article: Abdel-Wahab, F., El Menofy, N., El- Batal, A., Mosalam, F., Abdulall, A. Enhanced Antimicrobial Activity of the combination of Silver Nanoparticles and Different β Lactam antibiotics against Methicillin Resistant Staphylococcus aureus isolates. Azhar International Journal of Pharmaceutical and Medical Sciences, 2021; 1(1): 22-31. doi: 10.21608/aijpms.2021.53610.1017

DOI: 10.21608/aijpms.2021.53610.1017 https://aijpms.journals.ekb.eg/

failures of antimicrobial therapy and consequently higher rates of morbidity and mortality in addition to heath care costs [15]. A promising line for dealing with bacterial resistance is the antibiotics combination with AgNPs as they have excellent conformational entropy in polyvalent binding, which assists attachment to various function groups of antibiotics [16] .Several studies reveled that AgNPs potentiate the antimicrobial activity of antibiotics against resistant pathogen, in minute concentrations that reach to units of ppm, with no toxic effects on human cells [17]. The aim of the present investigation is to evaluate the antibacterial activity of plain AgNPs synthesized using Gamma-irradiation alone and in combination with different β -lactam antibiotics against methicillin resistant *Stahphylococcus aureus* isolates recovered from different clinical specimens by standard microbiological assays; disc diffusion method, broth microdilution assay in addition to measuring the dynamic growth curve of the bacteria in presense of AgNPs.

2. METHODS

2.1. Synthesis and characterization of silver nanoparticles

All synthesis and characterization techniques were conducted at National Center for Radiation Research and Technology (NCRRT, Cairo, Egypt). Silver nitrate ($AgNO_3$) and Polyvinylpyrrolidone (PVP) purchased from (Sigma-Aldrich St. Louis, MA, USA) were used for the synthesis of AgNPs. Briefly, solution of 1% PVP was mixed with 5.0 mM $AgNO_3$ and the mixture was exposed to gamma radiation at dose 20kGy at room temperature at facility practiced 60 (Co-Gamma chamber 4000-A-India) according to modified method described by El-Batal, et al. 2013, and El-Batal et al 2016 [18, 19].
After irradiation the produced AgNPs were characterized by UV-Visible absorption spectroscopy, dynamic light scattering technique in addition to transmission electron microscopy (TEM). [20]

2.1.1. *UV. Visible spectrophotometer determination*

For the initial determination of AgNPs, UV visible spectroscopy (JASCO-Japan-model V- 560) at a resolution of 1nm was done to measure the Surface Plasmon Resonance (SPR) for the wave length ranging from 100-800.

2.1.2. *Dynamic Light Scattering measurements (DLS)*

Average particle size and size distribution were detected by the dynamic light scattering (DLS) technique (PSS-NICOMP 380-ZLS, USA); 250µl of prepared solution were transferred to a one-use low volume cuvette, followed by equilibration to a temperature of 25ºC for 2 min, then five measurements were conducted using 12 runs of 10s each.

2.1.3. *Transmission Electron Microscopy Image (TEM)*

The particle size and surface morphology examinations of NPs were carried out using TEM (JEOL electron microscope JEM-100 CX) working at 80kV accelerating energy and SEM, (ZEISS, EVO-MA10, and Germany) Finally, EDX (BRUKER, Nano GmbH, D-12489, 410-M, Germany) was used to detect the elemental composition of AgNPs.

2.2. Isolation and identification of *Staphylococcus aureus*

Forty-five different clinical specimens including diabetic foot ulcer, burn ulcers and pus were supplied from microbiology labs in two hospitals (Al-Sayed Galal and Homiat Alabassia) in Egypt. The specimens were initially inoculated onto blood agar and mannitol salt ager plates (Oxoid® Limited, Basingstoke, UK) for isolation of *Staphylococcus spp.* The growing colonies were identified by Gram's staining and different biochemical tests including catalase, coagulase and DNAse etc. according to the identification scheme described by Mahon et al [21]. *Staphylococcus aureus* isolates resistant to cefoxitin 30µg were designated as MRSA. *S. aureus* ATCC® 25923 obtained from Egyptian Company for the Production of Sera and Vaccines (VACSERA, Dokki, Giza) was used as the quality control standard strain for antimicrobial susceptibility test.

2.3. Antimicrobial susceptibility testing and detection of MRSA isolates

Staphylococcus aureus isolates were investigated for their susceptibility to different antibiotic classes by the Kirby–Bauer disc diffusion assay according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [22]. Antibiotics discs were; Ampicillin 10µg, vancomycin 30µg, cefepime 30µg, cefotaxime 30µg, ceftazidime 30µg, cefoxitin 30µg ceftriaxone 30µg, imipenem 10µg and meropenem 10µg (Oxoid® Limited, Basingstoke, UK).

2.4. Antimicrobial susceptibility testing (AST) of AgNPs and silver nano-antibiotic combination by disc diffusion assay

Evaluation of antibacterial activity of silver nanoparticles colloid was performed by disc diffusion assay. Suspension of MRSA isolates was prepared in concentration of 1.5 x10^8 Cfu/mL and swabbed on the surface of Muller-Hinton agar plates (MHA) (Oxoid® Limited, Basingstoke, UK). Plain AgNPs discs were prepared by adding 10µl of AgNPs (500µg/ml) to 6 mm diameter Whatmman paper discs. For detection of the effect of silver nano-antibiotic combination, 10µl of AgNPs defined concentration were added to the standard discs of amoxicillin 30µg, ceftazidime 30µg, ceftriaxone 30µg, cefepime 30µg, cefotaxime 30µg, imipenem 10µg and meropenem 10µg. Discs soaked with 10µl silver nitrate (500µg/ml) and 10µl sterile distilled water were served as positive and negative controls discs respectively. Different discs were inoculated onto MHA plates and incubated at 37°C for 24 h. Inhibition zones were measured and compared to standard antibiotics inhibition zones published by CLSI, 2018 The disc diffusion assay was processed in triplicate [22].

The fold increase in the diameter of inhibition zone of each antibiotic after combination with AgNPs was calculated according to the equation; The fold increase = (b-a)/a x100, Where; (a) is the inhibition zone of antibiotic alone and (b) is the inhibition zone of antibiotic plus nanoparticles [23].

2.5. Determination of minimum inhibitory concentration of β-lactam antibiotics, silver nanoparticles solution and mixed solution of β-lactam antibiotics and silver nanoparticles against MRSA isolates

MIC was determined by broth microdilution assay in 96 multi-well microtiter plates according to the CLSI reference standards [22]. Commercially obtained β-lactam antibiotics; amoxicillin 1000mg, ceftazidime 1000mg, cefotaxime 1000mg, ceftriaxone 1000mg, cefepime 1000mg, imipenem 1000mg and meropenem 1000mg were used (EIPICO, El Sharqia, Egypt). One hundred microliter of double strength Muller-Hinton broth (MHB) (Oxoid® Limited, Basingstoke, UK) was distributed in all wells, a volume of 100µl from each aqueous antibiotic solution initially prepared (10240µg/ml) was pipetted into wells of the first row of microtiter plate, serial dilution was performed from the 1st to the 12th well to obtain concentrations ranged from 5120 to 2.5µg/ml. Finally, 10µl of 0.5 McFarland matched turbidity of freshly prepared bacterial suspension was added to each well. Two columns in each plate were used as positive and negative controls. Plates were wrapped and incubated at 37˚C for 18-24 h. The plates were examined visually against dark background for absence or presence of turbidity. The MIC was calculated as the smallest concentration where no visible bacterial growth was seen by comparison with controls. The results were interpreted according to CLSI reference standards. All the tests were run in duplicate and the average result was taken.

The antimicrobial activity of AgNPs (500µg/ml) was determined using the standard broth microdilution method as previously mentioned [24].

Mixed solutions of both Silver nanoparticles and antibiotics were prepared by dissolving; amoxicillin, imipenem and meropenem (10.24mg) in AgNPs solution (500µg/ml) the MICs of mixed solutions were determined using the standard broth micro dilution assay as previously mentioned, the concentrations run along the plate will ranged from 5120 +250 to 1.25+0.06µg/ml of different antibiotics and AgNPs respectively.

2.6. Growth kinetics of MRSA

Growth kinetic assay was performed according to Kasithevar et al [25]. Two MRSA colonies were inoculated in MHB medium and incubated overnight at 37˚C in shaking incubator adjusted at 200 rpm, 1 x 10^7 cells of the fresh bacterial suspensions were inoculated into conical flasks containing 100 mL fresh MHB media. To each flask, various concentrations of the AgNPs (125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.95 and 1 µg/mL) were added and incubated at 37 ˚C in shaking incubator adjusted at 200 rpm. A control flask (containing MHB without AgNPs) was kept along with samples. The growth kinetics was determined by measuring optical density (OD) at 600 nm at different time points of 1, 2, 3, 4, 6 and 24h of incubation using a UV-visible spectrophotometer.

3. RESULTS

3.1. Generation of AgNPs with desired properties.

In preparation of PVP-AgNPs, as the irradiation dose increases, the SPR band red shifted which reveal an increase in particles size. At gamma irradiation 20.0 kGy, a characteristic SPR band for PVP-AgNPs with absorbance showed maximum absorption (3.26) that obtained at the wavelength 440.0 nm. After the mixtures of PVP solution and $AgNO_3$ (5.0 mM) was exposed to 20.0 kGy gamma irradiation dose, deep brown color appeared indicating the formation of PVP coated silver nanoparticles (PVP-AgNPs) Figure 1. DLS is used to study the dispersal of the particles size and its outcomes were linked to the transmission electron microscopy (TEM) results. The average particle size of synthesized AgNPs was 27.1 nm in as noted by DLS technique Figure 2. Result of TEM confirmed

the spherical shapes of AgNPs within nano range from 9.5 nm to 28.6 nm with the mean diameter of 21.1 nm as showed in Fig. 3. The size of AgNPs received from DLS measures (27.1 nm) was greater than the TEM results (21.1 nm).

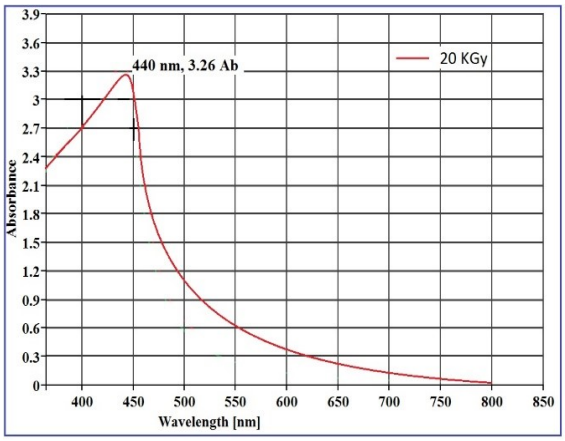

Figure (1): Uv-Vis. Spectroscopy of AgNPs synthesized by gamma irradiation (20.0 kGy) and PVP.

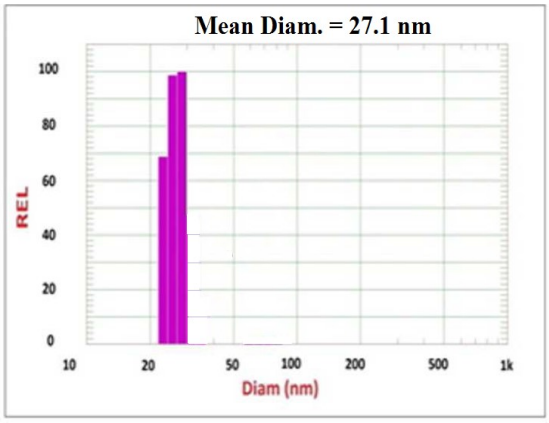

Figure (2): DLS of AgNPs synthesized by gamma irradiation (20.0 kGy) and PVP.

3.2. Isolation and identification of MRSA isolates

Eighty staphylococcus spp. were isolated out of 45 different clinical specimens; 45(56.2%) isolates were *Staphylococcus aureus* and 35(43.8 %) were coagulase negative staphylococcus spp. Out of 45 *Staphylococcus aureus* isolates, 21(46.6 %) isolates were identified as MRSA while 24(53.3%) were non-MRSA isolates (data not shown).

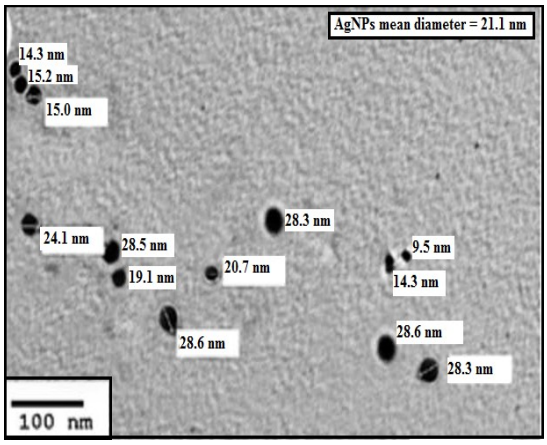

Figure (3): TEM image AgNPs synthesized by gamma irradiation (20.0 kGy) and PVP.

3.3. Minimum inhibitory concentrations and minimum bactericidal concentration of β- lactam antibiotics and AgNPs.

Minimum inhibitory concentrations and minimum bactericidal concentrations of β- lactam antibiotics against 21 MRSA isolates are listed in (table 1). The results showed that all isolates were full resistant to, amoxicillin, ceftazidime, cefotaxime, ceftriaxone, cefepime, while 3(14.2%) were sensitive to imipenem and meropenem with MIC ≤ 20µg/ml. Imipenem showed lowest bactericidal concentration against 2 MRSA isolates at concentration 80µg/ml and at 160µg/ml against 3 isolates. Plain AgNPs was evaluated for its antibacterial activity by disc diffusion method (5µg/disk) and broth microdilution assay (250µg/mL) (table 1 and 2) and revealed potential antibacterial activity. The range of inhibition zones was from 7.5 to 12 mm among MRSA isolates. Broth microdilution assay revealed that 8(38.1%) and 5(23.8%) were inhibited at conc. of 31.25 and 7.81 µg/mL of AgNPs respectively while it has bactericidal concentration at 125 and 62.5µg/mL for 11(52.3%) and 6(28.5%) of MRSA isolates respectively.

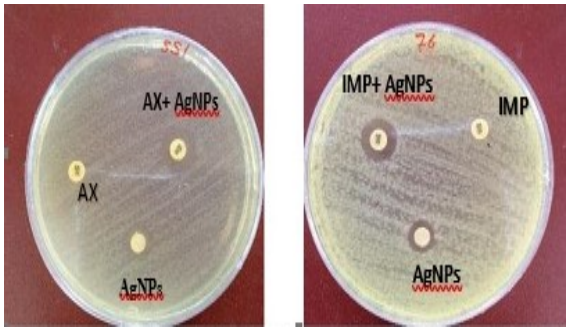

Figure (4): The synergistic effect between antibiotics (AX. Amoxicillin and IMP, Imipenem) and AgNPs obtained by disc diffusion method

3.4. The Antibacterial Efficacy of AgNPs/β-lactam antibiotics combinations

The enhancement of antibacterial activity of antibiotic discs (amoxicillin 30µg, cefepime 30µg, imipenem 10µg and meropenem 10µg) and AgNPs (5µg) was assessed as fold increasing in the imipenem/AgNPs and meropenem /AgNPs combinations with all MRSA isolates, the fold increase areas ranged between 33.3 to 100, 0 to 166.6 and 0 to 166.6 for aforementioned antibiotics respectively. For cefepime/AgNPs combinations the inhibition zone diameter by disc diffusion assay, the data are represented in table 2 and reveled the enhancement of activity of amoxicillin/AgNPs, fold increase area was ranged from 6.25 to 122.2 in 76.2% of MRSA isolates. Table 3 showed enhancement in activity and dropping of MIC values of amoxicillin/AgNPs, cefepime/AgNPs, meropenem/AgNPs and imipenem/AgNPs combinations. MIC of amoxicillin in amoxicillin/AgNPs mixture was decreased from 5120 µg/ml to reach 1.95 µg/ml in 28.5% of MRSA and 0.97 µg/mL in 19 % of MRSA isolates.

Table 1: Minimum inhibitory concentrations and minimum bactericidal concentration of β- lactam antibiotics and AgNPs against MRSA

Antibiotic Dilutions	Amoxicillin µg/mL		Ceftazidime µg/mL		Cefotaxime µg/mL		Ceftriaxone µg/mL		Cefepime µg/mL		Imipenem µg/mL		Meropenem µg/mL		AgNPs Dilutions	AgNPs µg/mL	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)		MIC (%)	MBC (%)
>5120	15 (71.4)	21 (100)	2 (9.5)	7 (33.3)	2 (9.5)	8 (38.1)	2 (9.5)	7 (33.3)	2 (9.5)	12 (57.1)	1 (4.7)	9 (42.8)	1 (4.7)	5 (23.8)	>250	0	0
2560	1 (4.7)	0	1 (4.7)	2(9.5)	1 (4.7)	0	1 (4.7)	1 (4.7)	0	0	1 (4.7)	0	1 (4.7)	0	125	2 (9.5)	6 (28.5)
1280	3 (14.2)	0	3 (14.2)	7 (33.3)	7 (33.3)	8 (38.1)	6 (28.5)	10 (47.7)	5 (23.8)	0	3 (14.2)	0	0	0	62.5	4 (19)	11 (52.3)
640	2 (9.5)	0	6 (28.5)	0	4 (19)	1 (4.7)	5 (23.8)	3 (14.2)	6 (28.5)	2 (9.5)	5 (23.8)	6 (28.5)	3 (14.2)	3 (14.2)	31.25	8 (38.1)	4 (19)
320	0	0	3 (14.2)	5 (23.8)	3 (14.2)	4 (19)	4 (19)	0	4 (19)	7 (33.3)	4 (19)	1 (4.7)	5 (23.8)	8 (38.1)	15.62	2 (9.5)	0
160	0	0	3 (14.2)	0	2 (9.5)	0	1 (4.7)	0	2 (9.5)	0	4 (19)	3 (14.2)	5 (23.8)	5 (23.8)	7.81	5 (23.8)	0
80	0	0	1 (4.7)	0	1 (4.7)	0	1 (4.7)	0	2 (9.5)	0	0	2 (9.5)	0	0	3.91	0	0
40	0	0	2 (9.5)	0	1 (4.7)	0	1 (4.7)	0	0	0	0	0	3 (14.2)	0	1.95	0	0
20	0	0	0	0	0	0	0	0	0	0	2 (9.5)	0	2 (9.5)	0	0.97	0	0
10	0	0	0	0	0	0	0	0	0	0	1 (4.7)	0	1 (4.7)	0	0.48828	0	0

3.5. Growth kinetics of MRSA

Treatment of standard inoculum of MRSA isolates with different concentrations of AgNPs ranged from 125 to 0.98 µg/ml was performed and growth was observed at different time interval. A higher fall in growth was observed with 125µg/ml AgNPs (Figure 5). It is obvious that the number of bacterial cells decreases, with higher concentration of of AgNPs and long exposure time. Moreover, there is no growth inhibition in positive control bacterial culture with no AgNPs addition.

4. DISCUSSION

High prevalence of global antibiotic resistance is alarming problem. *Staphylococcus aureus* is important pathogens that have growing rates of multi-antibiotic resistance profile in the last decades.

AgNPs is a powerful antimicrobial agent that assumed to act by different mechanisms including disruption of the cell wall, damage of cellular proteins, an increase in cell permeability and ultimately cell death [26]. The present study aimed to detect the enhancement of antibacterial activity of AgNPs in combinations with different members of β- lactam antibiotics against MRSA using various in vitro assays. AgNPs was prepared by Gamma-irradiation technique that produces AgNPs without any harmful effects. This process produces AgNPs with specific size, 9.5 nm to 28.6 nm with the average mean diameter of 21.1 nm that confirmed by TEM.

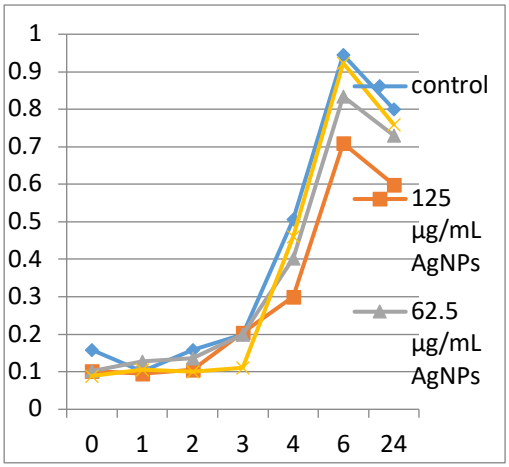

Figure (5): The growth kinetics of methicillin-resistant *Staphylococcus aureus* (MRSA) in the presence of different concentrations of AgNPs

Table 2: Mean Inhibition (mm) and Fold area increase of different antibiotics, silver nanoparticles and combined

MRSA isolates code	AgNPs MIZ	AX MIZ	AX + AgNPs M IZ	Fold area % [(b- a)/a] x 100	Cefipime M IZ	Cefipime + AgNPs M IZ	Fold area % [(b- a)/a] x 100	IMP MIZ	IMP + AgNPs MIZ	Fold area % [(b- a)/a] x 100)	MEM MIZ	MEM + AgNPs MIZ	Fold area % [(b- a)/a] x 100
SA01	**10**	**6**	**10**	**66.6**	**16**	**17**	**6.25**	**6**	**14**	**133.3**	**6**	**12**	**100**
SA02	**12**	**6**	**11**	**83.3**	**9**	**20**	**122.2**	**6**	**15**	**150**	**7**	**10**	**42.8**
SA03	**9**	**6**	**12**	**100**	**6**	**7**	**14.2**	**6**	**16**	**166.6**	**6**	**10**	**66.6**
SA04	**11**	**6**	**10**	**66.6**	**6**	**12**	**100**	**11**	**12**	**9.09**	**6**	**12**	**100**
SA05	**10**	**6**	**11**	**83.3**	**6**	**7**	**14.2**	**6**	**13**	**116.6**	**6**	**13**	**116.6**
SA06	**7**	**6**	**12**	**100**	**9**	**7**	**-22.2**	**6**	**14**	**133.3**	**6**	**14**	**133.3**
SA07	**10**	**6**	**8**	**33.3**	**6**	**7**	**14.2**	**10**	**10**	**0**	**6**	**14**	**133.3**
SA08	**11**	**6**	**10**	**66.6**	**6**	**7**	**14.2**	**6**	**11**	**83.3**	**6**	**14**	**133.3**
SA09	**10**	**6**	**11**	**83.3**	**6**	**7**	**14.2**	**6**	**12**	**100**	**8**	**12**	**50**
SA10	**11**	**6**	**10**	**66.6**	**12**	**10**	**-166**	**6**	**12**	**100**	**6**	**13**	**116.6**
SA11	**9**	**6**	**8**	**33.3**	**6**	**7**	**14.2**	**6**	**11**	**83.3**	**9**	**15**	**66.6**
SA12	**8**	**6**	**9**	**50**	**6**	**7**	**14.2**	**6**	**13**	**116.6**	**6**	**14**	**133.3**
SA13	**10**	**6**	**10**	**66.6**	**6**	**10**	**66.6**	**6**	**10**	**66.6**	**6**	**15**	**150**
SA14	**8**	**6**	**12**	**100**	**6**	**7**	**14.2**	**6**	**13**	**116.6**	**6**	**16**	**166.6**
SA15	**9**	**6**	**10**	**66.6**	**6**	**7**	**14.2**	**8**	**11**	**37.5**	**6**	**6**	**0**
SA16	**10**	**6**	**12**	**100**	**10**	**7**	**-30**	**6**	**12**	**100**	**6**	**13**	**116.6**
SA17	**9**	**6**	**10**	**66.6**	**7**	**8**	**14.2**	**6**	**12**	**100**	**6**	**13**	**116.6**
SA18	**12**	**6**	**9**	**50**	**12**	**7**	**-41.6**	**6**	**11**	**83.3**	**6**	**13**	**116.6**
SA19	**9**	**6**	**11**	**83.3**	**15**	**16**	**2.7**	**8**	**13**	**62.5**	**12**	**12**	**100**
SA20	**12**	**6**	**10**	**66.6**	**7**	**8**	**14.2**	**6**	**13**	**116.6**	**13**	**13**	**0**
SA21	**11**	**6**	**10**	**66.6**	**6**	**10**	**66.6**	**13**	**13**	**0**	**13**	**13**	**0**
***S. aureus* ATCC 25923**	**11**	**27**	**9**	**-66.6**	**23**	**18**	**-21.7**	**17**	**30**	**76.4**	**29**	**30**	**3.4**

antibiotics with silver nanoparticles by disc diffusion assay

MIZ: ***Mean Inhibition Zone AX, Amoxicillin, CAZ, Ceftazidime, IMP, Imipenem, MEM, Meropenem***

Table 3: Minimum inhibitory concentrations MICs of β- lactam antibiotics, AgNPs and their combination against MRSA isolates.

MRSA isolates code	AgNPs MIC µg/mL	AX MIC µg/mL	AX + AgNPs MIC	Cefipime MIC µg/mL	Cefipime + AgNPs MIC µg/mL	IMP MIC µg/mL	IMP + AgNPs MIC µg/mL	MEM MIC µg/mL	MEM + AgNPs MIC µg/mL
SA01	10.17	>5120	40+1.95	160	40+1.95	640	10+0.48	320	80+3.9
SA02	12.27	>5120	40+1.95	640	20+0.97	160	10+0.48	160	1.25+1.22
SA03	9.4	>5120	80+3.90	>5120	1280+62.5	>5120	1280+62.5	> 5120	640+31.25
SA04	11.17	>5120	80+3.90	>5120	640+31.2	2560	640+31.25	2560	40+1.95
SA05	10.3	1280	20+0.97	640	160+7.81	320	40+1.95	320	40+1.95
SA06	7.27	1280	10+0.48	320	160+7.81	160	80+3.90	160	20+0.97
SA07	10.33	640	20+0.97	640	320+15.6	320	20+0.97	320	10+0.48
SA08	11	>5120	160+7.8	1280	1280+62.5	640	10+ 0.48	320	80+3.9
SA09	10.33	>5120	40+1.95	1280	320+15.6	640	640+31.25	640	40+1.9
SA10	11.6	>5120	160+7.8	160	80+3.90	20	10+0.48	20	10+0.48
SA11	9.43	>5120	40+1.95	1280	1280+62.5	640	320+15.625	640	80+3.9
SA12	7.5	>5120	40+1.95	640	320+15.6	640	160+7.8	160	40+1.95
SA13	9.4	>5120	320+15.62	1280	1280+62.5	1280	640+31.25	320	80+3.90
SA14	8.3	>5120	160+7.81	1280	1280+62.5	1280	640+31.25	640	40+1.95
SA15	9	>5120	20+0.97	640	320+15.6	320	40+1.95	160	80+3.9
SA16	10.33	640	80+3.91	320	1280+62.5	160	640+31.25	40	80+3.9
SA17	8.5	>5120	40+1.95	320	1280+62.5	320	320+15.625	40	40+1.95
SA18	12.33	>5120	160+7.81	80	640+31.25	160	320+15.625	40	40+1.95
SA19	9.67	2560	80+3.9	80	20+0.9765	10	1.25+0.12	10	1.25+0.12
SA20	12	>5120	20+0.97	320	320+15.625	20	1.25+0.12	20	1.25+0.12
SA21	11.33	1280	80+3.9	640	320+15.625	1280	320+15.62	160	80+3.91
S. aureus ATCC 25923	≥1.5625	≥16	≤0.125+0.0122	≥16	≤0.125+0.012	≤0.125	≤0.125+0.012	≤0.125	≤0.125+0.01

The surface plasmon resonance of these AgNPs was recorded at a wavelength of 440 nm that ensure their small size.

In the present study, 21(46.6 %) isolates were identified as MRSA which less similar to study in same geographical area [27] that was about 24% of the total *S. aureus* isolates. The antibacterial sensitivity testing was done by Kirby–Bauer disc diffusion method using standard group of antibiotic discs and showed that all MRSA isolates were resistant to all tested antibiotic discs. *Staphylococcus* bacterial isolates resistant to three or more antibiotic groups or resistant to oxacillin were designated as multidrug-resistant *S. aureus* (MDRSA). [28] MRSA is the main cause for various infections and responsible for many community-acquired, epidemic and endemic nosocomial infections throughout the world [29]. MIC and MBC of AgNPs results of this study agreed with results obtained by other authors, which reported the powerful antimicrobial activity of colloidal silver nanoparticles against a wide range of microorganisms at a very low concentration, Kasithevar et al reported the antibacterial efficacy of green synthesized AgNPs against MRSA clinical isolates by modified Kirby-Bauer disc diffusion method and it were 19 mm at 100 µg/ml concentration of AgNPs., and 14 mm with 50 µg/ml AgNPs concentration. [30] Punjabi et al.[31] stated that higher concentration of AgNPs (5mg/ml) is required in order to be effective against *S. aureus.* In the same line Bankier et al [32] reported that 2.5 mg/mL showed moderate bactericidal activity against *S. aureus* ATCC 6538. our study reflects the enhanced antimicrobial activity of AgNPs against multidrug resistant clinical MRSA isolates. When comparing the results of AgNPs to other nanoparticles as copper and selenium we found that AgNPs was active at lower concentrations [33, 34]. The exact mode of action of AgNPs is still under investigation; however, a number of studies proposed that the antimicrobial activity of silver nanoparticles is related to free radicals formation that induced membrane damage [35].Additionally ions penetrate the cell membrane leading to the production of reactive oxygen species (ROS).

Furthermore silver ions inhibit vital enzymes and phosphorus-containing bases by interacting with their thiol groups, [36] it is likely that further damage could be caused by interactions with compounds such as the DNA. As well as they disrupt the cell wall formation in the bacteria, and cause damage to the cellular proteins [26, 37, 38]. preparing new Antibiotic combination with nano silver particles can be a valuable path for introducing new antibiotics to prevent a variety of hospital acquired infections [39]. In the current study the results of the fold increase areas were ranged between 33.3 to 100, 0 to 166.6 and 0 to 166.6 for amoxicillin/AgNPs, imipenem/AgNPs and meropenem /AgNPs combinations with all MRSA isolates, respectively. For cefepime/AgNPs combinations the fold increase area was ranged from 6.25 to 122.2 in 76.2% of MRSA isolates. Additionally most of the antibiotics upon combinations with AgNPs showed augmentation of antibacterial activity at concentrations far below the MIC of individual antibiotics by broth microdilution assay for example MIC of amoxicillin in amoxicillin/AgNPs mixture was decreased from 5120 µg/ml to reach 1.95 µg/ml in 28.5% of MRSA and 0.97 µg/mL in 19 % of MRSA isolates. Rahim and Mohammed suggested that ampicillin and cephalexin in combination with silver nanoparticles increase antibiotic efficacy by 5% and 25 % respectively [40]. Moreover in the current work bacterial inhibition was seen in lower concentrations of AgNPs especially with amoxicillin than reported by Surwade et al [39]. The observed enhancement of antibacterial activity could be due to the nanoparticle-antibiotic combination and not to the effect of AgNPs itself [41]. Combining AgNPs with antibiotics not only reduce the toxicity of both agents towards human cells but also augments their bactericidal properties [42]. The results of growth kinetics were in agreement with Kasithevar et al [25] who reported that the effect of AgNPs is dose dependent and viable bacterial number decrease at 80 µg/ml of AgNPs and commenced to increase in lower concentrations.

5. CONCLUSIONS

In the current work AgNPs have been synthesized using Gamma-irradiation, the synthesized AgNPs have showed effective bacterial inhibition against MRSA isolates. Furthermore, the growth kinetics revealed that number of bacterial cells decreases in a dose-dependent manner even at low concentrations. While it showed enhancement of antibacterial activity after nanoparticle-antibiotic combination by both Kirby Bauer disk diffusion method and broth microdilution assay against MRSA especially with amoxicillin, imipenem and meropenem so synthesis of potent antibacterial AgNPs against MRSA could act as a successful alternative line to the currently used antibiotics.

Acknowledgments: The authors thank Microbiology research Laboratory at Faculty of Pharmacy Al-Azhar for providing technical support and Microbiology laboratories in Al-Sayed Galal and Homiat Alabassia hospitals for providing clinical specimens.

Conflicts of interest

The authors declare no conflict of interest

Ethical statement: NA

Author contribution

NG and FB drafted the manuscript, FB and FM made the practical work, AK, AI and NG supervised the practical work and manuscript writing.

List of Abbreviations

MRSA: Methicillin resistant S. aureus; AgNPs: Silver nanoparticles; MIC: Minimum inhibitory concentration; $AgNO_3$: Silver nitrate; PVP: Polyvinylpyrrolidone; KGy: Kilogray; DLS: Dynamic light scattering technique; TEM: Transmission electron microscopy; SPR: Surface Plasmon Resonance; NCRRT: National Center for RadiationResearch and Technology; AST: Antimicrobial susceptibility testing; MHA: Muller-Hinton agar; MHB: Muller-Hinton broth; OD: Optical density; MDR: Multi-Drug Resistant

REFERENCES

1. Slama TG. Gram-negative antibiotic resistance: there is a price to pay. Critical Care. 2008; 12(4):S4.

2. Kerr KG, Snelling AM. Pseudomonas aeruginosa: a formidable and ever-present adversary. Journal of Hospital Infection. 2009; 73(4):338-44.

3. Chambers HF, DeLeo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nature Reviews Microbiology. 2009; 7(9):629.

4. Hemlatac B A., Antibacterialactivity of silver nanoparticles conjugated withantibiotics. Bionano Frontier. 2014; 7(2):32-5.

5. Huh AJ, Kwon YJ. "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. Journal of controlled release. 2011; 156(2):128-45.

6. El-Batal A, Haroun BM, Farrag AA, Baraka A, El-Sayyad GS. Synthesis of Silver Nanoparticles and Incorporation with Certain Antibiotic Using Gamma Irradiation. 2014.

7. Ashour AA, Raafat D, El-Gowelli HM, El-Kamel AH. Green synthesis of silver nanoparticles using cranberry powder aqueous extract: characterization and antimicrobial properties. Int J Nanomedicine. 2015; 10:7207-21.

8. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004; 275(1):177-82.

9. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. Journal of colloid and interface science. 2004; 275(1):177-82.

10. Cui L, Chen P, Chen S, Yuan Z, Yu C, Ren B, et al. In situ study of the antibacterial activity and mechanism of action of silver nanoparticles by surface-enhanced Raman spectroscopy. Analytical chemistry. 2013; 85(11):5436-43.

11. Xu H, Qu F, Xu H, Lai W, Wang YA, Aguilar ZP, et al. Role of reactive oxygen species in the antibacterial mechanism of silver nanoparticles on Escherichia coli O157: H7. Biometals. 2012; 25(1):45-53.

12. Lara HH, Ayala-Núñez NV, Turrent LdCI, Padilla CR. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World Journal of Microbiology and Biotechnology. 2010; 26(4):615-21.

13. Li W-R, Xie X-B, Shi Q-S, Duan S-S, Ouyang Y-S, Chen Y-B. Antibacterial effect of silver nanoparticles on Staphylococcus aureus. Biometals. 2011; 24(1):135-41.

14. Petri W. Penicillins, cephalosporins, and other β-lactam antibiotics. Goodman and Gilman's the pharmacological basis of therapeutics 8th ed New York, NY: McGraw-Hill. 1990:1477-504.

15. Kong KF, Schneper L, Mathee K. Beta-lactam antibiotics: from antibiosis to resistance and bacteriology. Apmis. 2010; 118(1):1-36.

16. El-Batal A, Haroun BM, Farrag AA, Baraka A, El-Sayyad GS. Synthesis of silver nanoparticles and incorporation with certain antibiotic using gamma irradiation. Journal of Pharmaceutical Research International. 2014:1341-63.

17. Panacek A, Smekalova M, Kilianova M, Prucek R, Bogdanova K, Vecerova R, et al. Strong and Nonspecific Synergistic Antibacterial Efficiency of Antibiotics Combined with Silver Nanoparticles at Very Low Concentrations Showing No Cytotoxic Effect. Molecules. 2015; 21(1):E26.

18. El-Batal A, El-Baz A, Abo Mosalam F, Tayel A. Gamma irradiation induces silver nanoparticles synthesis by Monascus purpureus. J Chem Pharm Res. 2013; 5(8):1-15.

19. El-Batal AI, Gharib FAE-L, Ghazi SM, Hegazi AZ, Hafz AGMAE. Physiological responses of two varieties of common bean (Phaseolus vulgaris L.) to foliar application of silver nanoparticles. Nanomaterials and Nanotechnology. 2016; 6:13.

20. Basavaraja S, Balaji S, Lagashetty A, Rajasab A, Venkataraman A. Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium semitectum. Materials Research Bulletin. 2008; 43(5):1164-70.

21. Mahon CR, Lehman, D.C. and Manuselis, G. Textbook of Diagnostic Microbiology. third edition ed: Elsevier; 2007.

22. Wayne P. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 28th informational supplement. CLSI document M100-S20. 2018.

23. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomedicine: Nanotechnology, Biology and Medicine. 2010; 6(1):103-9.

24. Xu N, Cheng H, Xu J, Li F, Gao B, Li Z, et al. Silver-loaded nanotubular structures enhanced bactericidal efficiency of antibiotics with synergistic effect in vitro and in vivo. Int J Nanomedicine. 2017; 12:731.

25. Kasithevar M, Periakaruppan P, Muthupandian S, Mohan M. Antibacterial efficacy of silver nanoparticles against multi-drug resistant clinical isolates from post-surgical wound infections. Microbial pathogenesis. 2017; 107:327-34.

26. Yamanaka M, Hara K, Kudo J. Bactericidal actions of a silver ion solution on Escherichia coli,

studied by energy-filtering transmission electron microscopy and proteomic analysis. Applied and environmental microbiology. 2005; 71(11):7589-93.

27. Ahmed EF, Gad GF, Abdalla AM, Hasaneen AM, Abdelwahab SF. Prevalence of methicillin resistant Staphylococcus aureus among Egyptian patients after surgical interventions. Surgical Infections. 2014; 15(4):404-11.

28. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection. 2012; 18(3):268-81.

29. Fischbach MA, Walsh CT. Antibiotics for emerging pathogens. Science. 2009; 325(5944):1089-93.

30. Kasithevar M, Saravanan M, Prakash P, Kumar H, Ovais M, Barabadi H, et al. Green synthesis of silver nanoparticles using Alysicarpus monilifer leaf extract and its antibacterial activity against MRSA and CoNS isolates in HIV patients. Journal of Interdisciplinary Nanomedicine. 2017; 2(2):131-41.

31. Punjabi K, Mehta S, Chavan R, Chitalia V, Deogharkar D, Deshpande S. Efficiency of biosynthesized silver and zinc nanoparticles against multi-drug resistant pathogens. Frontiers in microbiology. 2018; 9:2207.

32. Bankier C, Matharu R, Cheong Y-K, Ren G, Cloutman-Green E, Ciric L. Synergistic antibacterial effects of metallic nanoparticle combinations. Scientific reports. 2019;9(1):1-8.

33. DeAlba-Montero I, Guajardo-Pacheco J, Morales-Sánchez E, Araujo-Martínez R, Loredo-Becerra G, Martínez-Castañón G-A, et al. Antimicrobial properties of copper nanoparticles and amino acid chelated copper nanoparticles produced by using a soya extract. Bioinorganic chemistry and applications. 2017; 2017.

34. Chaudhary NK, Mishra P. Bioinorganic Chemistry and Applications. Article ID. 2017; 6927675:13.

35. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine. 2007; 3(1):95-101.

36. Hatchett DW, White HS. Electrochemistry of sulfur adlayers on the low-index faces of silver. The Journal of Physical Chemistry. 1996; 100(23):9854-9.

37. Rai MK, Deshmukh S, Ingle A, Gade A. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. Journal of applied microbiology. 2012; 112(5):841-52.

38. Kim K-J, Sung WS, Moon S-K, Choi J-S, Kim JG, Lee DG. Antifungal effect of silver nanoparticles on dermatophytes. J Microbiol Biotechnol. 2008; 18(8):1482-4.

39. Surwade P, Ghildyal C, Weikel C, Luxton T, Peloquin D, Fan X, et al. Augmented antibacterial activity of ampicillin with silver nanoparticles against methicillin-resistant Staphylococcus aureus (MRSA). The Journal of antibiotics. 2019; 72(1):50-3.

40. Rahim KAAA, Mohamed AMA. Bactericidal and antibiotic synergistic effect of nanosilver against methicillin-resistant Staphylococcus aureus. Jundishapur journal of microbiology. 2015; 8(11).

41. Birla S, Tiwari V, Gade A, Ingle A, Yadav A, Rai M. Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. Letters in Applied Microbiology. 2009; 48(2):173-9.

42. Selvarani M. Investigation of the synergistic antibacterial action of copper nanoparticles on certain antibiotics against human pathogens. Int J Pharm Pharm Sci. 2018; 10:83-6.