Original Research Article

Impact of Different Chemically Synthesized Silver nanoparticles on Nosocomial Infection

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ABSTRACT

Aim: Nosocomial infection (NI), or so-called hospital-acquired infection, is one of the most important problems facing us and the world at this time. The aim of this study is to investigate the effect of minimum inhibition concentration of Ag-NPs on isolated bacteria from nosocomial infections.
Place and Duration of Study: Gastroenterology Surgical Center, Mansoura University, Mansoura, Egypt, between July 2017 and July 2018.
Methodology: A total of 368 different samples of 100 patients were taken which staying for 3 days or more in Gastroenterology Surgical Center, Mansoura University, Egypt. Three different types of Ag-NPs synthesized by three different chemical methods were used.
Results: The results showed that the highest rate of infection of NI was found in *K. Pneumoniae* with an estimated percentage of 40%. The size of spherical Citrated-Ag-NPs was found in range of 15-57 nm. On contrary, the size of spherical Polyvinyl-Pyrrolidon (PVP) and PVP Glucose Ag-NPs were found to be smaller with size ranging from 7.8-23 and 7.58-25 nm, respectively.
Conclusion: Our findings showed that PVP Glucose Ag-NPs had the highest impact on all types of isolated bacteria associated with nosocomial infections.

Keywords: Nosocomial infections, Ag-NPs , *K. pneumoniae*, MRSA, *E. coli*.

INTRODUCTION:

Nosocomial infections (NIs) can be characterized as infection that were obtained amid hospitalization by patients in whom the disease was absent or incubating at the time of entrance [1]. Indeed, the danger of nosocomial infections in intensive care units is ten multiple times more prominent than those gained in general medical and surgical wards [2].National Healthcare Safety Network and Center for Disease Control for observation have arranged nosocomial infections locales into 13 types, with 50 infection destinations, which are explicit based on biological and clinical criteria. Among these, respiratory tract infection and surgical site infections, urinary tract infection in addition to Blood Stream Infection [3]. Advancement in the antibiotic treatment of bacterial infections has impressively diminished mortality from numerous irresistible illnesses [4]. Microorganisms in the ordinary human flora touchy to the given medication are smothered, while resistant strains persevere and may end up endemic in the healing center [5]. Antibiotics are at times ending up less powerful as a result of resistance [6].As an antibiotic turns out to be broadly utilized microorganisms impervious to this medication in the long run rise and may spread in the health care setting [7]. Excessive and improper utilization of expansive range antibiotics, particularly in medicinal services settings are hoisting nosocomial infection [8]. Penicillin-resistant pneumococci, multidrug-resistant tuberculosis, MRSA, vancomycin-resistant *S. aureus* (VRSA) are regular instances of medication resistant bacteria [9]. This issue is especially basic in developing nations where progressively costly second-line antibiotics may not be accessible or moderate [10]. Bacteria create resistance when they get new genetic material. Poor antibiotic recommending chooses for resistant bacteria. The hereditary material that encodes resistance is exchanged to different strains [11]. Progressions in nanotechnology have prompted the improvement of nanoparticles with exceptional physiochemical properties and functionalization and can conquer limitations presented by ordinary antimicrobial agents [12]. Ag-NPs

can inhibit the activities of interferon gamma and alpha tumor necrosis factor which are engaged with inflammation. The anti-inflammatory impacts initiated by Ag-NPs anyway make it an astounding possibility for use as anti-inflammatory agents that can be utilized for different treatments [13]. In the studies completed in the most recent years, silver was characterized as "oligodynamic" because of its capacity to deliver a bactericidal impact at low fixations. This component of Ag ions emerges from its high partiality towards DNA, RNA, proteins, catalysts and etc. because of the reaction with functional groups, for example, phosphate, carboxylate, thiol, hydroxyl, imidazole, indole or amines what can meddle in microbial procedures[14]. Mechanism of silver incited cell death in which silver may disturb various bacterial cell forms, including metabolism, disulfide bond formation and iron homeostasis [15]. Small Ag-NPs or Ag^+ can enter the microbial body causing the harm of its intracellular structures [16]. As a result ribosomes might be denatured with inhibition of protein synthesis, just as interpretation and translation can be hindered by the binding with the genetic material of the bacterial cell [17]. Different appearances like auxiliary changes in the cell membranes and creation of small electron dense granules framed by silver and sulfur have been prove and loss of DNA replication ability [18], or intercalate themselves among pyrimidine and purine and denature the DNA molecule [19]. Ag-NPs can physically associate with the cell surface of different bacteria. This is especially essential on account of gram negative bacteria where various investigations have watched the adhesion and aggregation of Ag-NPs to the bacterial surface [20].

The penetrability of bacterial membranes causing efflux of reducing sugars and proteins just as the exhaustion of the levels of intracellular adenosine triphosphate (ATP) [21]. Besides, Ag-NPs can scatter the proton motive force of bacteria. Disposal of bacterial proton motive force results in cell demise [22]. This impact is exceedingly affected by the Ag-NPs ' size, shape and concentration [23] and an investigation utilizing *E. coli* affirmed that Ag-NPs aggregation on the cell membrane makes holes in the integrity of the bi-layer which lead to a penetrability increment lastly bacterial cell passing [24].

Among the existed revealed techniques, up until now, chemical techniques are favored for the synthesis of Ag-NPs because of the straightforwardness in created them in solution. Chemical synthesis procedure of the Ag-NPs in solution for the most part utilizes the accompanying three principle segments: (a) metal precursors, (b) reducing agents and (c) stabilizing/capping agents. Moreover, the creating and geometry of Ag-NPs depend on the nucleation and resulting stacking of the Ag-cores [25]. The development of colloidal solutions from the reduction of silver salts includes two phases of nucleation and ensuing growth. It is additionally uncovered that the size and the shape of synthesized Ag-NPs are unequivocally reliant on these stages [26].

MATERIALS AND METHODS

Identification of bacteria

Six different types of bacteria have been identified by VITEK 2 compact 15 (Biomerieux, France), and they were causal *E. coli*, *K. pneumoniae*, MRSA, *P. mirabilis*, *Ps. aeruginosa* and *Ps. putida*. Interestingly, different synthetized Ag-NPs were diluted serially from 10^{-1} to 10^{-5}. Next, a variety of cultured nutrient agar plates were then inoculated with 120 µL of each diluted antibiotic according to agar well-diffusion method [27].

Polyvinyl Pyrrolidon (PVP) Ag-NPs

PVP Ag-NPs were prepared by adding 0.5 ml of 30mM of trisodium citrate to 50 mL dist. water with continuous stirring. Then, 1 mL of 5 mM of $AgNO_3$ was added. Next, 0.5 mL of 50 mM of freshly prepared $NaBH_4$ was added to the aforementioned mixture. As a result, the colour of suspension was turned into yellow immediately. Finally and after 30 seconds, 0.5 mL of 1 mM of PVP was added. As a consequence, the colour of suspension changed into dark yellow after 30 min.

PVP Glucose Ag-NPs (PVP Glu Ag-NPs)

PVP Glu Ag-NPs were synthesized according to Kittler et al. [28] by dissolving 2 g of glucose and 1 g of PVP (M.wt 40000 g/mole) in 40 mL dist. water heated to 70°C under continuous stirring. Then, 0.5 g of $AgNO_3$ was dissolved in 1 mL of triple dist. water and added quickly. The suspension was kept at 70°C for 30 min. and then allowed to reach room temperature. The typical yield with respect to Ag was about 5%.

Citrated Ag-NPs (Cit Ag-NPs)

Cit Ag-NPs were synthesized according to Van Dong et al. [29] by heating 50 mL of 1mM of $AgNO_3$ to its boiling point under continuous stirring. A total of 5 ml of 1% of sodium citrate was then

added to $AgNO_3$ drop by drop. Next, the solution was allowed to reach room temperature.

Ultraviolet Visible (UV-Vis)

Surface plasmon resonance peaks (SPR) by UV-Vis extinction spectra were recorded using a spectrophotometer (CARY 60, Agilent Technologies, Australia) in absorption mode (200-800 nm) at desired dilutions of Ag-NPs colloids.

Transmission Electron Microscopy (TEM)

The size and morphology of Ag-NPs was determined by TEM 2100 (Tokyo- Japan), Mansoura university, at an operational voltage of 200 kv with 15 magnification.

Ag-NPs Antimicrobial Susceptibility Test

Antimicrobial susceptibility of Ag-NPs diffusion synthesis was investigated. The Kirby-Bauer diffusion method was used as antimicrobial susceptibility testing method. Different types of Ag-NPs were diluted serially from 10^{-1} to 10^{-5}. Next, a variety of cultured nutrient agar plates were then inoculated with 120 µL of each diluted antibiotic according to agar well-diffusion method [30].

RESULTS AND DISCUSSION

Indeed, progressions in nanotechnology have prompted the improvement of nanoparticles with exceptional physiochemical properties and functionalization and can conquer limitations presented by ordinary antimicrobial agents [12]. Silver nanoparticles are increasingly used in various fields, including medical, food and health care purposes, due to their unique physical and chemical properties including electrical, thermal, high electrical conductivity, and biological properties [21, 31]. Ag-NPs are widely known for its antimicrobial properties against microbes such as bacteria, fungi, and virus [32]. Due to their proven antimicrobial properties, Ag-NPs are widely used in the daily used commercial products, such as plastics, food packaging, soaps, pastes, food, and textiles, which has increased their market value to a great extent. Therefore, this work is concerned with investigating the effect of minimum inhibition concentration of Ag-NPs on isolated bacteria from nosocomial infections.

First of all, all bacterial strains isolated were tested for antibiotic sensitivity by standardized disk-diffusion method on nutrient agar medium. Fourteen antibiotics were used (OXOID, England) Ciprofloxacin, Norfloxacin, Ofloxacin, Levofloxacin, Nitrofurantoin, Meropenem, Imipenem, Cefotaxime, Amoxicillin, Ampicillin+Sulbactam, Amikacin, Neomycin, Erythromycin and Ceftriaxone as presented in Table 1.

Three-hundred and sixty-eight samples were incorporated in this work. These samples were categorized into four different groups. They are urine (n=100), stool (n=100) and sputum (n=100) samples in addition to surgical wounds (n=68). These samples were further classified based on nosocomial infections into positive and negative groups as shown in Table 2. The UV-Vis absorption spectra of samples were presented in Figure 1. All the samples present the characteristic surface Plasmon of Ag-NPs. The size of spherical Cit Ag-NPs was found in range of 15-57 nm. On contrary, the size of spherical PVP Ag-NPs and PVP Glu Ag-NPs were found to be smaller with size ranging from 7.8-23 and 7.58-25 nm, respectively.

In the present study, the effect of PVP Glu Ag-NPs *per se* was estimated on isolated bacteria at different dilutions as provided in Table 3. As for the other two types of Ag-NPs, they did not give positive results with isolated bacteria**.**

As a result, our findings depicted that the MICs for *E. coli*, *P. mirabilis*, *K. pneumoniae* and MRSA were 10^{-2} while those of *Ps. aeruginosa* and *Ps. putida* MICs were 10^{-3}. Consistent to our findings, Jain et al. [33] has found that Ag-NPs have been shown to be definitely an effective antibiotic against *E. coli* and *S. aureus*. One pioneering study was performed to analyze the interactions of Ag-NPs with *Ps. putida* biofilms. The results suggested that biofilms are impacted by the treatment with Ag-NPs [34]. PVP Ag-NPs showed good antibacterial activity towards *S. aureus*, *E. coli* and *Ps. aeruginosa* [35]. Ghazvini et al. [36], Lara et al. [37] and Ahmadi et al. [38] also proved the bactericidal efficacy of Ag-NPs on *Ps. aeruginosa*. It was reported that the most bactericidal potential of Ag-NPs against MRSA, but only moderate activity was observed against *K. pneumonia* [39]. Chudasama et al. [40] indicated that Ag-NPs have antibacterial impact against *E. coli*, *Shigella*, *Proteus vulgaris* and *S. aureus*, respectively. Lu et al. [41] reported that PVP Ag-NPs have the best antimicrobial activity against *E. coli* and *Fusobacterium nuceatum*. Interestingly, The capability of AgNPs to inhibit bacteria is due to the interaction between Ag-NPs and the bacteria membrane once they are in contact with the organism. Their interaction causes the bacteria membrane damage, which then leads to bacteria cellular death [42]. It is also possible via the oxidation of the silver nanoparticles

to silver ions producing reactive oxygen species. In this particular mechanism, the toxicity properties are due to the involvement of disruption of the mitochondrial respiratory chain by Ag-NPs leading to the production of ROS, which in turn causes bacteria DNA damage [43]. Alternatively, it is possibly due to their nanoparticles surface charge. For instance, Ag-NPs having a negative charge on their surface are less toxic compared with those with a positive charge [44]. Indeed, the morphology of the obtained Ag-NPs does not change significantly with size. Smaller Ag-NPs appear to have a better ability to enter into microorganisms. Truth be told, the interactions with the membranes and any subsequent harm, which may prompt cell death, are surely increasingly apparent on account of Ag-NPs with littler diameter and a positive zeta potential [45].

Guzman et al. [30] indicated that Cit Ag-NPs with diameter 9 and 11 nm have antibacterial activity against *E. coli* and *Ps. aeruginosa* with clear zone diameter 10 mm and *S. aureus* and MRSA with clear zone diameter 12 mm. Raza et al. [46] proved that Cit Ag-NPs and PVP Ag-NPs have antibacterial effect against *Ps. aeruginosa* and *E. coli* and the PVP Ag-NPs were the highest impact. Interestingly, Spherical citrate-stabilized nanoparticles carried a strongly negative charge (zeta potential -30 mV; hydrodynamic diameter 85 nm). PVP-stabilized nanoparticles carried a less negative charge (zeta potential -17 mV; hydrodynamic diameter 85 nm). The rate of dissolution and the final degree of dissolution were higher for PVP-stabilized nanoparticles than for citrate-stabilized nanoparticles [28].

Table 1. Effect of antibiotics against isolated bacteria according to agar disk diffusion

Name of Antibiotics	Clear zone (mm)					
	E. coli	*K. pneumoniae*	*P. mirabilis*	MRSA	*Ps. aeruginosa*	*Ps. putida*
Ciprofloxacin (CIP)	27	R	33	31	29	14
Norfloxacin (NOR)	28	R	38	31	25	R
Ofloxacin (OFX)	32	R	31	40	30	13
Levofloxacin (LEV)	32	R	30	40	R	16
Nitrofurantoin (F)	23	R	10	38	R	12

Meropenem (MEM)	32	R	29	15	35	31
Imipenem (IPM)	29	R	23	40	25	24
Cefotaxime (CTX)	30	R	15	R	R	R
Amoxicillin (AX)	16	R	R	R	R	R
Ampicillin+Sulbactam (SAM)	R	R	R	R	R	R
Amikacin (Ak)	22	9	21	27	24	20
Neomycin (N)	19	11	20	20	19	R
Erythromycin (E)	R	R	R	R	R	R
Ceftriaxone (CRO)	27	R	14	R	R	R

(R) Resistant.

Table 2. Types and percentages of appearance of pathogenic bacteria isolated from different patient samples associated with nosocomial infection.

Bacteria	Urine (n=35) [a]		Stool (n=28) [a]		Sputum (n=27) [a]		Surgical wounds (n=50) [a]	
	n	%	n	%	n	%	n	%
E. coli	10	28.5	10	35.7	5	18.5	3	6
K. pneumonia	14	40	13	46.43	8	29.36	21	42
P. mirabilis	3	8.6	5	17.9	0	0	3	6
MRSA	3	8.6	0	0	14	51.9	15	30
Ps. Aeruginosa	3	8.6	0	0	0	0	3	6
Ps. Putida	2	5.7	0	0	0	0	5	10

[a] n: Total number of positive samples.

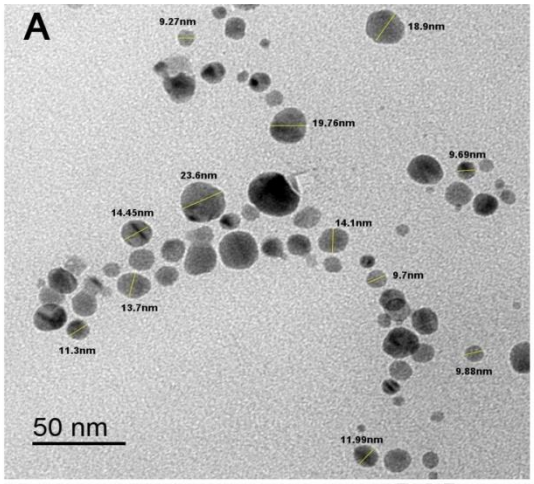
Table 3. Effect of PVP Glu Ag-NPs at different concentrations on isolated bacteria

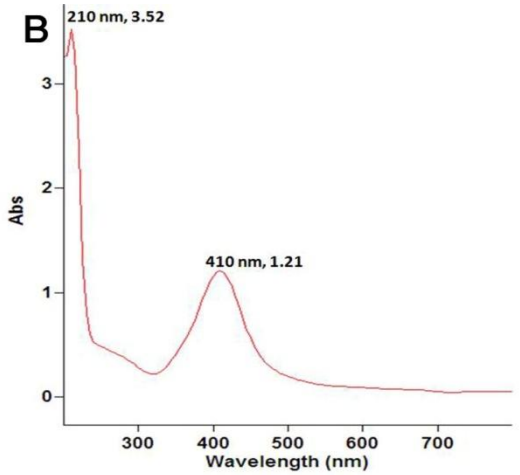
Dilutions	***E. coli*** Clear zone (mm)	***K. pneumoniae*** Clear zone (mm)	***P. mirabilis*** Clear zone (mm)	**MRSA** Clear zone (mm)	***Ps. aeruginosa*** Clear zone (mm)	***Ps. putida*** Clear zone (mm)
Stock	16	18	19	19	25	19
10^{-1}	14	15	16	17	23	17
10^{-2}	13*	14*	14*	15*	18	16
10^{-3}	R	R	R	R	12*	12*
10^{-4}	R	R	R	R	R	R
10^{-5}	R	R	R	R	R	R

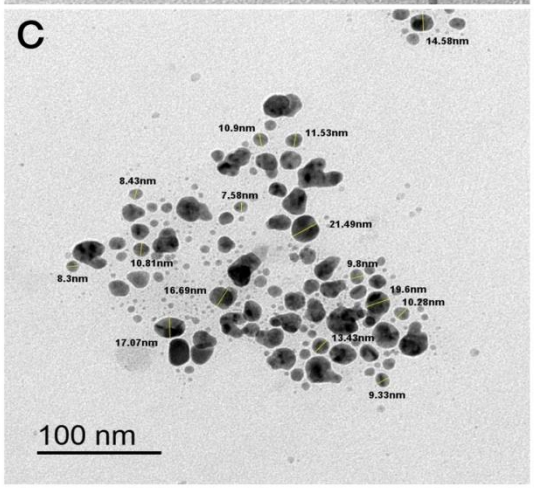
(*) MIC and (R) Resistant.

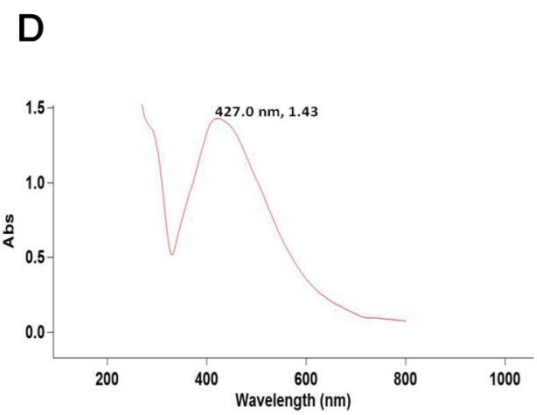
Figure 1. Shape, size and surface plasmon resonance peaks (SPR) of Ag-NPs by TEM and UV-Vis.

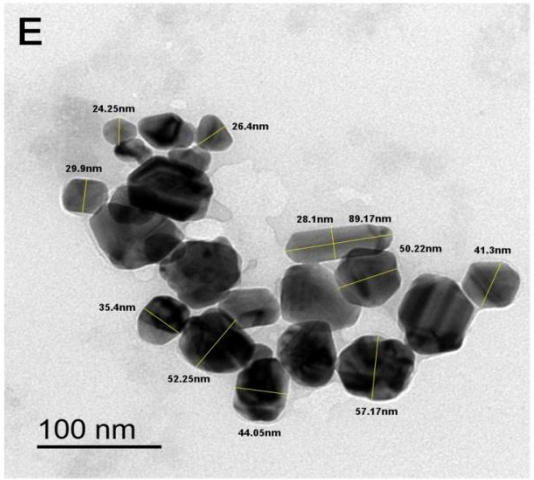
(A) Shape and size of PVP-Ag-NPs by TEM. (B) SPR of PVP-Ag-NPs by UV-Vis. (C) Shape and size of PVP-Glu-Ag-NPs by TEM. (D) SPR of PVP-Glu-Ag-NPs by UV-Vis. (E) Shape and size of Cit-Ag-NPs by TEM. (F) SPR of Cit-Ag-NPs by UV-Vis.

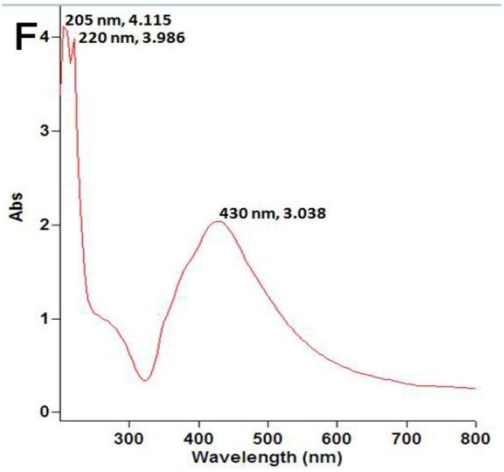












CONCLUSION

Our findings showed that PVP Glu Ag-NPs with size ranging from 8.3-21.49 nm with average 12.517 nm had the highest impact when compared with PVP Ag-NPs with size ranging from 9.27-19.76 nm with average 14.031 nm and Cit-Ag-NPs with size ranging from 24.25-57.7 nm with average size of 40.975 nm which had no effect on all types of isolated bacteria associated with nosocomial infections. This may be due to the accumulation of nanoparticles or negative charge around PVP Ag-NPs and the decrease ~~of~~ release of silver ions. However, inactivity of Cit Ag-NPs is probably due to the large size and also ~~to~~ the multiple forms formed (circular, circular, pyramidal). Thus, silver nanoparticles are considered potential agents to help manage and prevent infections and can be used in several applications against bacteria resistant to common antibiotics or even multi-resistant bacteria. Additionally, this work showed the potential of using silver nanoparticles as an alternative to conventional antimicrobial agents that are currently used. This may give a clue of using silver nanoparticles combined with antibiotics to enhance antimicrobial activity.

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