Original Research Article

Impact of Different Chemically Synthesized Silver nanoparticles on Nosocomial Infection

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-gNPs were found to be smaller with size ranging from 7.8-23 and 7.58-25 nm, respectively.
Conclusion: Our findings showed that PVP-Glu-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:

By and large, 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, 2]. 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 [3].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 [4]. Advancement in the antibiotic treatment of bacterial infections has impressively diminished mortality from numerous irresistible illnesses [5]. 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 [6]. Antibiotics are at times ending up less powerful as a result of resistance [7].

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 [8]. Excessive and improper utilization of expansive range antibiotics, particularly in medicinal services settings are hoisting nosocomial infection [9]. Numerous strains of Staphylococci, Pneumococci, Enterococci and tuberculosis are presently impervious to most or all antimicrobials which were once successful. Multi-resistant *Klebsiella* and *Ps. aeruginosa* are predominant in numerous healing centers [1]. Penicillin-resistant pneumococci, multidrug-resistant tuberculosis, MRSA, vancomycin-resistant *S. aureus* (VRSA) are regular instances of medication resistant bacteria [10]. This issue is especially basic in developing nations where progressively costly second-line antibiotics may not be accessible or moderate [11]. 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 [12]. Progressions in nanotechnology have prompted the improvement of nanoparticles with

exceptional physiochemical properties and functionalization and can conquer limitations presented by ordinary antimicrobial agents [13]. 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 [14]. 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[15]. Mechanism of silver incited cell death in which silver may disturb various bacterial cell forms, including metabolism, disulfide bond formation and iron homeostasis [16]. Small Ag-NPs or Ag^+ can enter the microbial body causing the harm of its intracellular structures [17]. 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 [18]. 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 [19], or intercalate themselves among pyrimidine and purine and denature the DNA molecule [20]. 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 [21].

The penetrability of bacterial membranes causing efflux of reducing sugars and proteins just as the exhaustion of the levels of intracellular adenosine triphosphate (ATP) [22]. Besides, Ag-NPs can scatter the proton motive force of bacteria. Disposal of bacterial proton motive force results in cell demise [23]. This impact is exceedingly affected by the Ag-NPs ' size, shape and concentration [24] 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 [25].

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 [26]. 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 [27]. 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 [28].

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*. and in turn kept for subsequent analysis for assessing the influence of different Ag-NPs on them. Interestingly, different 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 [29].

Polyvinyl-Pyrrolidon (PVP) Ag-NPs

PVP-Ag-NPs were prepared according toVan Dong et al. [30] by adding 0.5 ml of 30mM of tri sodium citrate (TSC) in 50 ml triple dist. water under continuous stirring, then add 1 ml of 5 mM of $AgNO_3$. Stirring was stopped, then add freshly prepared 0.5 mL of 50 mM of $NaBH_4$ quickly, Colour of suspension turned on a light yellow immediately. After 30 seconds, 0.5 ml of 1 mM of PVP was added. The suspension changed to dark yellow colour after reaction had proceeded for another 30 min.

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

PVP-Glu-Ag-NPs were synthesized by reduction with glucose in the presence of PVP according to Kittler et al. [31] by dissolving 2 gm of glucose and 1 gm of PVP in 40 ml triple dist. Water, heated to 70°C under continuous stirring. Then 0.5 gm 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 cooled to room temperature. Final colour of suspension was brownish.

Citrated-Ag-NPs (Cit-Ag-NPs)

Cit-Ag-NPs were synthesized according to Van Dong et al. [30] by heating 50 mL of 1mM of $AgNO_3$ to its boiling point under continuous stirring. A solution of 1% of sodium citrate (5 ml) was then added drop by drop. The reaction was allowed to take place until the colour change to a greenish yellow solution. The solution was leaved to cool in 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 were 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 [32].

RESULTS AND DISCUSSION

First of all, all bacterial strains isolated were tested for antibiotic sensitivity by standardized disk-diffusion method on nutrient agar medium according to Collee et al. [33]. Fifteen antibiotics were used (OXOID, England) Ciprofloxacin, Norfloxacin, Ofloxacin, Levofloxacin, Nitrofurantoin, Meropenem, Imipenem, Cefotaxime, Amoxicillin, Ampicillin+Sulbactam, Amikacin, Neomycin, Erythromycin, Ceftriaxone and Vancomycin 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 . PVP-Glu-Ag-NPs present a narrow band with a maximum at 427 nm. PVP-Ag-NPs has narrow band, which presents a maximum at 410 nm. Cit-Ag-NPs has a narrow band, which presents a maximum at 430 nm. 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 Ag-NPs *per se* was estimated on isolated bacteria at different concentrations as provided in Table 3.

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. [34] 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 [35]. PVP-Ag-NPs showed good antibacterial activity towards *S. aureus*, *E. coli* and *Ps. Aeruginosa* [36]. Ghazvini et al. [37]; Lara et al. [38] and Ahmadi et al. [39] 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* [40]. Chudasama et al. [41] indicated that Ag-NPs have antibacterial impact against *E. coli*, *Shigella*, *Proteus vulgaris* and *S. aureus*, respectively. Lu et al. [42] reported that PVP-Ag-NPs have the best antimicrobial activity against *E. coli* and *Fusobacterium nuceatum*. 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 [43].

Guzman et al. [32] 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. [44] 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.

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. pneumoniae	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.

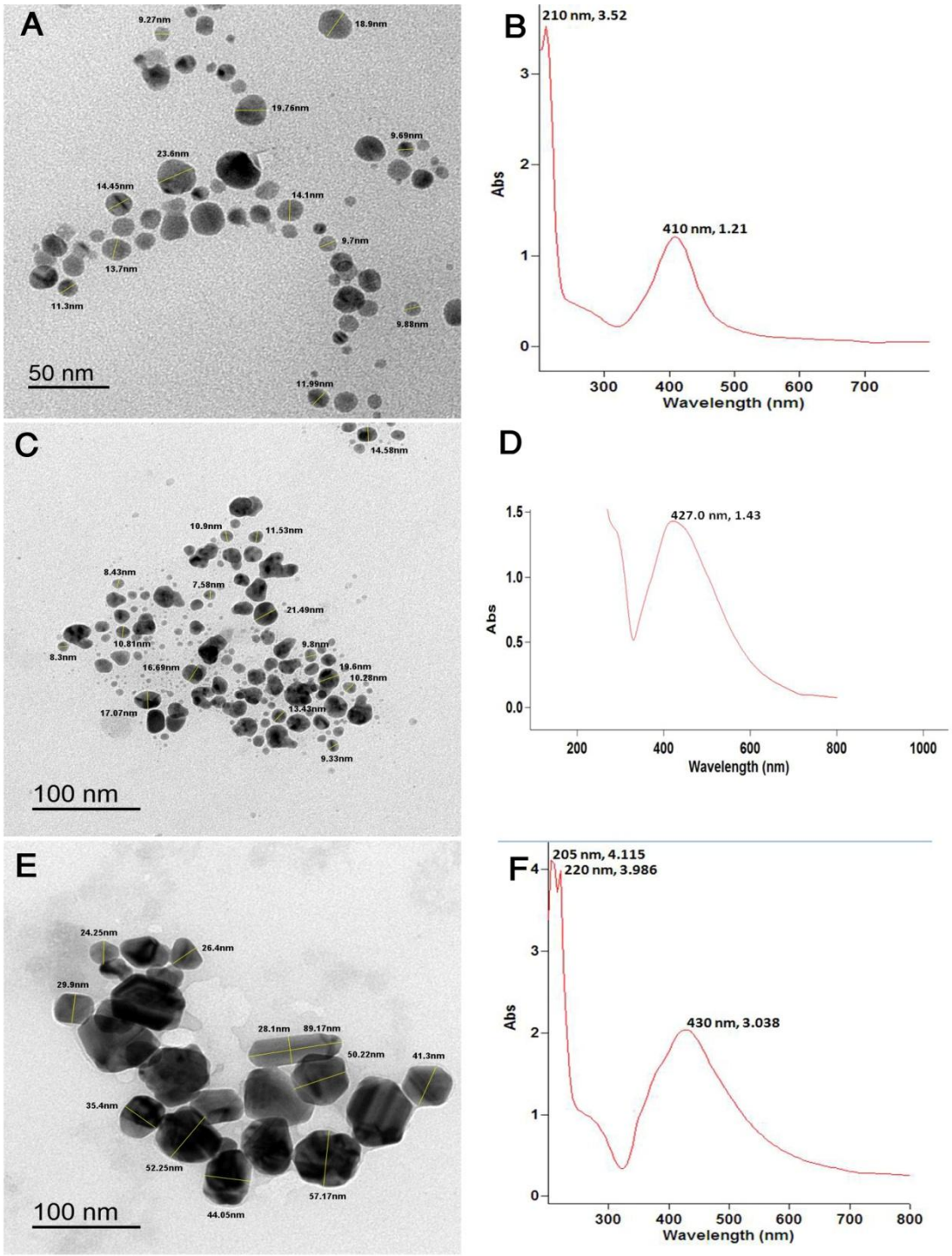
Table 3. Effect of Ag-NPs at different concentrations on isolated bacteria

Concentration	***E. coli***	***K. pneumoniae***	***P. mirabilis***	**MRSA**	***Ps. aeruginosa***	***Ps. putida***
	Clear zone (mm)	Clear zone (mm)	Clear zone (mm)	Clear zone (mm)	Clear zone (mm)	Clear zone (mm)
Stock	16	18	19	19	25	19
$\mathbf{10^{-1}}$	14	15	16	17	23	17
$\mathbf{10^{-2}}$	13*	14*	14*	15*	18	16
$\mathbf{10^{-3}}$	R	R	R	R	12*	12*
$\mathbf{10^{-4}}$	R	R	R	R	R	R
$\mathbf{10^{-5}}$	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 had the highest impact on all types of isolated bacteria associated with nosocomial infections.

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