Evaluation and Comparison the Antibacterial Activity of Silver Nano Particles (AgNPs) and Silver Nitrate (AgNO3) on Some Pathogenic Bacteria

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Abstract

Resistance of bacteria to some antibiotics increased tremendously therefore demanding for solutions and new medicine becomes essential as this problem related to human health. For this purpose, using of nanoparticles increased rapidly during the last years. In this study, the effect of silver nanoparticles (AgNPs) and silver nitrate (AgNO3) on bacteria had been evaluated and compared. Commercial purchased silver nanoparticles (with the particles size 20nm and Scanning Electron Microscopy (SEM) image) were characterized by X-ray diffraction (XRD). Antimicrobial activity of nanoparticles Silver and silver nitrate were evaluated on some pathogenic bacteria including samples including: Streptococcus pyogenes and Staphylococcus aureus (Gram Positive Bacteria)and Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Acine to bacter baumannii and Vibrio cholerae (Gram Negative Bacteria).In this research, antibacterial activity for nanoparticle silver and silver nitrate at different concentrations started from 10 to 150μg ml−1 against bacterial isolates was measured by agar diffusion technique and Minimum Inhibitory Concentration (MIC)/ Minimum bactericidal Concentration (MBC).These pathogens caused different hazardous infections and some of these bacteria are multi-drug resistant, the results exhibited remarkable antibacterial activity of silver nanoparticles (AgNPs) compared to moderate effect of silver nitrate (AgNO3) against most tested isolates including serious Gram Negative Bacteria resistant bacteria K.pneumonia, S. typhi and V. cholearae , the exception was A. baumannii as these bacteria didn’t affected definitely by any concentrations of Ag NPs or AgNO3 in the detection tests of MIC and MBC.

Keywords: Silver Nan particles, Silver Nitrate, Pathogenic Bacteria

Introduction

Antibiotic resistance is one of the world's most essential product for public healthcare problems and almost any changes of microbes has grown to be stronger and less exposed to antibiotic treatment, intimidating new strains of infectious diseases or super strains that are equally more complicated to heal and response, in addition to that, Drug-resistant bacteria which are raising pathogens whose resistance profiles at hand a foremost challenge for containing their spread and collision on human health[1].

Silver ions and silver nanoparticles also have inhibitory and lethal effects on bacterial species such as E. coli S. Aureus and even yeast [2-3-4].Among metal nanoparticles with proven antimicrobial activity, those made of silver are particularly effective bactericidal agents [5].

Ag-NPs have distinctive physio-chemical properties such as surface-enhanced Raman scattering, chemical stability, high electrical and thermal conductivity, catalytic activity in addition to that nonlinear optical behavior [6] on the other hand, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity [7] besides silver has lower possibility for development of microbial resistance against it [8].

Silver has always been used for treatment various diseases; anciently , it found to use as an antiseptic and antimicrobial agent against Gram-positive and Gram-negative bacteria[9-10] as a result of its low cytotoxicity [11] .Silver in the nano size range has emerged as the most invested nano-antimicrobial for production of materials such as medical implants, water disinfection applications,
surgical coatings, dietary supplements, food packaging, cosmetics and textiles[12-13].

As a result of all mentioned above and because nowadays silver nanoparticles (AgNPs) have attracted much attention in the scientific researches, this investigation aimed to evaluate and compare the antibacterial activity of silver as silver nanoparticles (AgNPs) and silver nitrate(AgNo3) as well as Ag-NPs toxicity could be correlated with the particle size [14-15]and because the size of the particle plays a central role in antimicrobial activity [14 ]therefore the size of (AgNPs) used in this study was 20 nm .

Materials and Methods

Bacterial Samples

Seven bacterial samples consisted of Streptococcus pyogenes and Staphylococcus aureus (Gram Positive bacteria) and Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Acine to bacter baumannii and Vibrio cholera (Gram Negative bacteria)were included in this study. These bacteria were isolated from patients didn’t receive any antibiotic treatment before collecting specimens and including sputum, pus, urine, stool and sore throat of patients admitted different wards in Marjan Hospital/ Hilla city/ Babylon Province / Iraq on May 2016, which were sent to the microbiology laboratory for routine culture identification and sensitivity testing.

Further identification tests performed for all bacterial isolates including biochemical tests, culture and preserving of isolates were used according to [16-17]. The bacterial samples were maintained in the brain, heart infusion broth containing 15% glycerol at -75 °C during the research period.

Silver Nanoparticles (Ag) NP and Silver Nitrate (AgNo3)

In all experiments of this research, silver nitrate as solution (AgNO3,100 g mol−1, ultrapure water (UP water, pH 5.660.1) and silver nanoparticles with grain size (18-20) nm and spherical, metal basis, 99.99%were purchased commercially from company (Hong international group ltd).

Determination Biological Activity of Silver Nanoparticles and Silver Nitrate by Agar Diffusion Method

To evaluate and compare the antimicrobial activity of silver nanoparticles and silver nitrate in vitro against all bacterial samples by the agar diffusion technique, both of them was tested along on the same agar plate, silver nanoparticles powder was suspended in water for achievement the interaction of the silver nanoparticles with the bacteria, in this experiment several concentrations of silver nanoparticles (20, 40,60, 80 and 100 μg ml−1) were tested against each type of eight bacterial samples collected.

The inoculums size was adjusted so as to deliver final inoculums of approximately 108 colony forming unit (CFU)/ml from the grown bacterial culture of a 24 hr- old for all strains to compare the turbidity of each sample to the 0.5 McFarland standards, the broth of these microorganisms were culture on Mueller–Hinton (MH) medium agar plates. After solidification of 25 ml nutrient agar in Petri plates, hollows of six wells (5 millimeter diameter) were cut into the agar by cork borer then all the collected pathogenic bacteria samples were tested on this agar. 0.1 ml of nano silvers solutions with the concentrations prepared earlier were applied in five wells while the last one filled with 0.1ml of silver nitrate mol−1, the inoculums size was adjusted so as to deliver final inoculums of approximately 108 colony forming unit (CFU)/ml, compared with the turbidity of a sample of the 0.5 McFarland standards, all Petri dishes were incubated at 5-8 °C for 2-3 h to permit good diffusion and then incubated for 24 h at 37 °C. The assessment of antibacterial was based on measuring the diameter of the inhibition zone (mm) formed around the well. Furthermore, the control including plates without silver nanoparticles and without silver nitrate, then Plates were incubated for 24 h at 37C[18-19].

Determination of Minimum inhibitory concentration and Minimum Bactericidal concentration (MIC/ MBC) as antimicrobial activity of Silver Nanoparticles and Silver Nitrate

The antimicrobial activities of nano silver - sized particles and silver nitrate( for comparison) were evaluated, antimicrobial activity against all bacterial strains (8 strains) performed by serial dilution method according to[20]with some modification in order to determine the minimum inhibitory concentration (MIC and MBC) in the culture broth. In the current research, a serial dilution of silver nanoparticles and silver nitrate started from 10 to 150μg ml−1 of prepared with each of them (Ag NP, and AgNo3) used for determination of the.
minimum inhibitory concentration (MIC) values. MIC values were taken as the lowest concentration required to suppress the growth of the bacteria in the test tube after incubation (showed no turbidity) while the minimum bactericidal concentration (MBC) was determined by sub culturing 50 μl from each test tube showing no apparent growth (clear), if there was no growth this concentration was taken as MBC. [20]. 100 μl of the bacterial cells adjusted to (1x10^6 ml^-1) were added to Mueller–Hinton (MH) medium broth, then 10 μl from each of the serially diluted solutions of the compounds( Ag NPs and AgNO₃) were added to the bacterial cells.

The MIC was defined as the lowest concentration required for inhibiting visible growth of bacteria in the test tube after incubation (showed no turbidity) while the minimum bactericidal concentration (MBC) determined by sub culturing of 50 μl from each test tube showing no apparent growth (clear), if there was no growth this concentration was taken as MBC.

**Result and Discussion**

**Antibacterial Activity**

It is known that silver has antimicrobial properties therefore it has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to the host cells [21-22-23]. The present study aimed to compare the antibacterial activity between commercially purchased silver nanoparticles with the size of 20 nm and silver nitrate against all isolated bacteria.

Seven pathogenic bacteria samples isolated from patients admitted to different wards in Marjan Hospital/ Hilla city/ Babylon Province / Iraq on May 2016 included in this research :S. pyogenes and S.aureus (Gram Positive Bacteria)and E.coli, K. pneumonia, S. typhi, A. baumannii and V. cholerae (Gram Negative Bacteria), Table (1).

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- S.pyogenes</td>
<td>Sore throat</td>
</tr>
<tr>
<td>2- S.aureus</td>
<td>Pus</td>
</tr>
<tr>
<td>3- E.coli</td>
<td>Urine</td>
</tr>
<tr>
<td>4- K.pneumonia</td>
<td>Sputum</td>
</tr>
<tr>
<td>5- S. typhi</td>
<td>Stool</td>
</tr>
<tr>
<td>6- A.baumannii</td>
<td>Urine</td>
</tr>
<tr>
<td>7- V.cholerae</td>
<td>Stool</td>
</tr>
</tbody>
</table>

Evaluation of antibacterial effect of both (Ag NPs and AgNO₃) was performed by two methods: Agar well diffusion method and Minimum inhibitory concentration and Minimum Bactericidal concentration (MIC/ MBC).

The results of this work exhibited that Ag NPs (20 nm particle sized) had noticeable effectiveness compared with AgNO₃ against all bacterial isolates. As shown in Table (2) and Fig. (1), the results of Agar diffusion method indicated that S. pyogenes and S. aureus affected by Ag NPs and had convergent inhibition zones at all concentrations used including highest concentration (100μg/ml). About Gram negative bacteria, V. cholerae possessed the largest inhibition zone in treating with Ag NPs, but the most remarkable inhibition zone was for A. baumannii (one of the most important no socomial pathogen and multidrug resistant bacteria), this bacterium didn’t affected by any concentration of Ag NPs or AgNO₃ definitely tested in the current research.
Table 2: Diameter of inhibition zone (mm) of Silver Nano particles (Ag NP$\_20$ nm) against bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>20 µg/ml</th>
<th>40 µg/ml</th>
<th>60 µg/ml</th>
<th>80 µg/ml</th>
<th>100 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S.\ pyogenes$</td>
<td>12</td>
<td>17</td>
<td>17</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>$S.\ aureus$</td>
<td>10</td>
<td>13</td>
<td>15</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>$E.\ coli$</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>$K.\ pneumonia$</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>$S.\ typhi$</td>
<td>8</td>
<td>9</td>
<td>9</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>$A.\ baumannii$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$V.\ cholerae$</td>
<td>13</td>
<td>15</td>
<td>17</td>
<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>

Fig 1: Schematic Diagram of inhibition zone (mm) of Silver Nano particles (Ag NP, 20 nm) against bacterial isolates

Silver nitrate (Ag$\text{NO}_3$) also used in this study but showed less antibacterial activity in the Agar diffusion method compared with Ag NP$\_s$ against tested bacteria Table (3) and Fig.(2) . Most significant result here was that most of bacterial isolates didn’t affected with Ag NP$\_s$ at the lower concentrations (20,40 and 60 µg/ml) and the biological activity started by a concentrations of 80 µg/ml, except for two isolates including $V.\ cholerae$ because only this bacteria affected early at a concentrations of 40 µg/ml and had the inhibition zone of 4mm . Other isolate was $S.\ pyogenes$ which affected at a concentrations of 60 µg/ml with inhibition zones of 9 mm). In addition to that, $V.\ cholerae$ had the largest inhibition zone (15mm) in the Agar diffusion method of (Ag$\text{NO}_3$) amongst all investigated bacteria including Gram positive and Gram negative bacteria, while $K.\ pneumonia$, and $A.\ baumannii$ didn’t affected decisively by silver nitrate at any concentrations also with 100 µg/ml.

Table 3: Diameter of inhibition zone (mm) of Silver Nitrate against bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>20 µg/ml</th>
<th>40 µg/ml</th>
<th>60µg/ml</th>
<th>80 µg/ml</th>
<th>100µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S.\ pyogenes$</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>$S.\ aureus$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>$E.\ coli$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
In comparison to the effect of silver nitrate (AgNo3), lowest MIC was for V. cholera (70 µg/ml which was the concentration of MBC also) but the magnificent MIC of (AgNo3) was for K. pneumonia and E.coli (140 µg/ml and130µg/ml respectively).

Interestingly, A. baumannii also didn’t affected definitely by Ag NPs or AgNo3 at any concentrations in the detection tests of MIC and MBC.

Table 4; Antimicrobial activity MIC/MBC (µg/ mL) of Silver nanoparticles(Ag NPs 20 nm) & Silver Nitrate (AgNo3) against Bacterial isolates

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Silver Nano particles</th>
<th>Silver Nitrate</th>
<th>AgNo3 Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
</tr>
<tr>
<td>S.pyogenes</td>
<td>50 µg/ml</td>
<td>60 µg/ml</td>
<td>80 µg/ml</td>
</tr>
<tr>
<td>S.aureus</td>
<td>60 µg/ml</td>
<td>60 µg/ml</td>
<td>80 µg/ml</td>
</tr>
<tr>
<td>E.coli</td>
<td>70 µg/ml</td>
<td>90 µg/ml</td>
<td>130µg/ml</td>
</tr>
<tr>
<td>K.pneumonia</td>
<td>70 µg/ml</td>
<td>100 µg/ml</td>
<td>140µg/ml</td>
</tr>
<tr>
<td>S. typhi</td>
<td>70 µg/ml</td>
<td>90 µg/ml</td>
<td>110µg/ml</td>
</tr>
<tr>
<td>A.baumannii</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>V.cholerae</td>
<td>50 µg/ml</td>
<td>50 µg/ml</td>
<td>70 µg/ml</td>
</tr>
</tbody>
</table>
Generally, the treatment of *A. baumannii* infections are often difficult because of their widespread resistance to the major groups of antibiotic and have the propensity to tolerate drying [24] In particular, *A. baumannii* has become important organism in the Intensive Care Unit (ICU)[25], urinary tract, surgical wounds, respiratory, peritoneum, skin and eyes infections [24]. All tested isolates are important pathogenic bacteria but concerning *V. cholerae*, usually cholera is epidemic in Iraq like incidence last year.

Cholera remains a serious public health problem for some developed countries although primarily affecting developing countries [26]. The Eltor biotype was transmitted from Asia and Middle East to Iraq and Iran in 1965 – 1966 through trading in the seventh pandemic [27]. In this investigation, *V. cholerae* widely affected by Ag NP, and to lower extent with AgNo but also *V. cholerae* still highly affected with Ag NP, or AgNo3 compared with other bacterial strains tested here in this study.

Studies on *V. cholerae* demonstrated the massive damage of the membrane with collapse of the proton motive force induced by low concentrations of free silver ions [28] and that in coordinate with the results of the present study. The inhibitory effect of silver ions caused by other cause may be related to different concentrations, other important refer to two bacterial samples tested (*S. aureus* and *S. typhimurium* in the mentioned study while in the current study six samples in addition to *S. aureus* were tested.

Generally, size, shape and specific surface area are important factors that improve the silver nanoparticle toxicity to bacteria [33-34] .Silver nanoparticles with different shaped (e.g., spherical, rod shaped, and truncated triangular) inhibited *E. coli* differentially [34].

The antibacterial properties were related to the nanoparticles total surface area [36] smaller particles with larger surface to volume ratios have greater antibacterial activity, similar results were published by [37].Small particles exhibited higher several biological events such as attachment to cell membranes, changing the membrane permeability through adsorption to the negatively charged bacterial cell wall, inactivating the cellular enzymes and production of Reactive Oxygen Species (ROS) in addition[29].

The results of this research are in agreement with the study of [30] specially they investigated Ag-NPs with the size 20 nm (such as in this research) and they founded that these Particles were cytotoxic to *E. coli* and TEM analysis indicated the complete disruption of the bacterial membrane after few minutes in contact with silver nanoparticles, and that agree with the result of the present study in the distinct activity against *E. coli* [31].

The results of this study partially in agreement with the results of study [32] their investigation indicated that the AgNO and Ag-NPs in PEG suspension gave high and similar antibacterial activity against Gram-negative and Gram-positive bacteria while in current study the antibacterial activity of Ag-NPs higher than that of AgNO and that may be because they use Ag-NPs with the size range around 10–25 nm but the size of Ag-NPs in this study was 20 nm.or may be because they used only disk diffusion method not well diffusion agar or dilution such as in this study antimicrobial activity than big particles; this can be due to high particle penetration when these particles have smaller sizes.

The antibacterial properties are referred to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity [38-39].

Silver nanoparticles have been evaluated for their antimicrobial activities against a wide range of pathogenic organisms [40-41]. The highest sensitivity was observed against Methicillin resistant *Staphylococcus aureus* (MRSA) followed by Methicillin resistant *Staphylococcus epidermidis* (MRSE) and *S. pyogenes*, while moderate antimicrobial activity was observed in case of the Gram negative pathogens *S. typhi* and *K.*
pneumonia[42] and these results in agreement with results of the present research. On the other hand, AgNPs have been shown to be definitely an effective antibiotic against S. epidermidis, S. aureus, E. coli and S. typhi [43].

Several studies indicated that AgNP activity is strongly dependent on the size [44-45]. In fact, it was found that the bactericidal activity of AgNPs of smaller dimensions (<30 nm) is optimal against S. aureus and K. pneumoniae [46]. Increasing scientific evidence has demonstrated that AgNP activity would depend not only on their concentration and size [47-48], but also on their shape [49].

Concerning the shape of nanoparticles, truncated-triangular nanoparticles appeared to be more effective for microbial killing; however, spherical nanoparticles are still considered to be the best-suited for practical applications in either colloidal form or immobilized state [50-51-52] therefore in this research spherical Ag-NPs had been investigated for antibacterial activity.

Notably, Ag-NPs introduce broad-spectrum biocidal activity towards many different microbial strains and this antibacterial effect was found to be size as well as dose dependent. AgNPs antibacterial activity greatly enhanced as their size was reduced from 100 nm to 20 nm [53].

Worth mentioning, it is not logical to compare all MIC/MBC values reported in different researches with others for several reasons such as the large variation in various factors involved in the antibacterial studies, like variation in initial bacterial concentration, composition of culture media and microbial strains, specific study concluded that for a particular sized of AgNPs, the MIC/MBC values were exhibited variation for different microbial strains and as a result indicate the strain-specificity in antibacterial activity [54] in addition, the size, shape, preparation methods of silver nanoparticles is likely to play a crucial role and may cause variation in the antibacterial effect [52-34-55].

Analysis Results of Silver Nan Particles (Ag NPs):

Scanning Electron Microscopy (SEM):

Image (1) shows silver nanoparticles (Ag NPs). The results of this analysis showed the highly homogeneity distribution of Ag NPs and spherical shape. According to manufacture product specification data sheet, the particle size was 20 nm.

X-ray Diffraction Analysis (XRD)

From the X-ray test Figure (3) of silver nanoparticles at a diffracted angle (20° to 80°), a crystalline peak appeared which indicate crystalline structure at (2θ 38.0992). This indicates that crystalline material is prepared and were still in accordance with the standards silver nanoparticles XRD which agrees with the

Image 1: SEM Result of Silver Nan particles
results of [56-57]. In the current research the calculation of the grain size of silver nanoparticle was in the range (18-20)nm. According of this results the peaks indicated that the main composition of nanoparticles was silver and clearly no obvious other peaks present as impurities were found in the XRD patterns, therefore, this introduces obvious evidence for the presence of Ag-NPs.

Conclusion

The current research indicated that Ag NPs had noticeable effectiveness compared with AgNO₃ against all bacterial isolates tested, the future research may study biochemical or genetics changes occurs in the bacterial cells after treating with nano silver, study the effect of Ag NPs with different sizes particularly smaller than 20 nm, study the effect of Ag NPs with different shapes, investigate antibacterial activity in the combination of nano silver and free ions and examine some other pathogenic bacteria species.

More researches and studies required related to effect of Nano silver against A. baumannii cause it exhibited extremely highly resistance compared with other tested bacteria. At the same time, the extraordinary inhibition of V. cholerae with Nan particles of silver introduce a spark for interfering Nano silver in some techniques related with water treatment and filtration, especially cause cholera is endemic disease in Iraq, last epidemic of cholera occurred in Iraq in the final part of last year (2015).

From the results obtained it is suggested that silver-nanoparticles could be used effectively against some multidrug resistant and hazardous bacteria also could be used in safety environmental and medical applications particularly in the hospitals.
furniture, operation room, stricter of some devices like catheters to prevent or reduce bacterial colonization and microbiological labs as no socomial infections take place there. In addition to that, silver nano particles, may be utilized in medical fields but certainly in the limitations related to human health and toxicity of nano silver side effects, studies of the American Biotech Labs have concluded that nanosilver products are not toxic to cells, animals or humans[58] with low concentration of nanosilver solutions there is no toxic effects on human’s tissues were reported [59].

References


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