

## Comparison of the Antibacterial Effects of Nanosilver With 18 Antibiotics on Multidrug Resistance Clinical Isolates of *Acinetobacter baumannii*

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**Background:** *Acinetobacter baumannii* plays a significant role in nosocomial infections. Multidrug-resistant *A. baumannii* is recognized to be among the most difficult antimicrobial-resistant Gram-negative bacilli to be controlled and treated. For this reason we examined the efficacy of nanosilver material with strong disinfectant properties against different types of bacteria.

**Objectives:** The purpose of this study was to examine the antibacterial activity of silver nanoparticles and compare them with some antibiotics against clinically isolated *A. baumannii* bacteria.

**Materials and Methods:** 60 *A. baumannii* strains were isolated from clinical specimens of university hospitals in Tehran province of Iran. The susceptibility test against 18 different antibiotics was investigated by disk diffusion methods. Colloidal nanosilver with about 6-34 nm particle size was prepared by chemical method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) effect of nanosilver on *A. baumannii* were assessed at serially diluted nanosilver concentrations and compared to different antibiotics.

**Results:** The findings showed a 100% resistance of *A. baumannii* to different antibiotics, including piperacillin, cefixime, ceftazidime, ceftizoxime, cephempim and clavulanat, and more than 90% resistance against imipenem, cefotaxime, ceftriaxone, ciprofloxacin and cotrimoxazole and lower resistance was seen against nitrofurantoin (62.8%), gatifloxacin (56.85%), levofloxacin (55.18%), gentamycin (29.32%), piperacillin/tazobactam (13.34%), meropenem (13.16%) and amikacin (12.07%). MIC and MBC mean value of nanosilver dilutions were determined as 27.34 ppm and 54.68 ppm, respectively. All isolates (100%) were susceptible to more than MIC of nanosilver in contrast showed high resistance to multiple classes of antibiotics.

**Conclusions:** We conclude that nanosilver is a strong bactericidal agent to Multidrug resistant *A. baumannii* bacteria, so it can be useful to prevent a variety of nosocomial infections.

**Keywords:** *Acinetobacter baumannii*; Antibacterial; Emotions; MBC

### 1. Background

The genus *Acinetobacter* includes Gram-negative coccobacilli, with a DNA G+C content of 39 to 47 mol%, which are strictly aerobic, nonmotile, catalase positive and oxidase negative and has been divided into 16 genome species (1, 2). *Acinetobacter* species are capable of colonizing and infecting human beings. They have been involved in a variety of nosocomial infectious diseases. *Acinetobacter baumannii* has become a particularly important organ-

ism in the Intensive Care Unit (ICU) (3). Infections occur in the respiratory and urinary tract, peritoneum, surgical wounds, skin and eyes. Bacteremia and secondary meningitis with mortality rates from 20% to 27% were seen in several cases (1). They have been reported to be the common agents of nosocomial pneumonia, particularly ventilator-associated pneumonia (VAP) in hospital ICUs (3, 4). *A. baumannii* were shown to have the propensity to tolerate drying and resist multiple classes of antibiotics and

#### Implication for health policy/practice/research/medical education:

*Acinetobacter baumannii* play a significant role in nosocomial infections. Multidrug-resistant *A. baumannii* is recognized to be among the most difficult antimicrobial-resistant Gram-negative bacilli to control and treat. For this reason we examined the efficacy of nanosilver material with strong disinfectant properties against different types of bacteria. This Paper was the result of research on nanosilver products for prevention and treatment of some infectious disease.

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these are the key factors enabling the organism to survive and spread in the nosocomial environment. In general, the treatment of *A. baumannii* infections are often extremely difficult because of their widespread resistance to the major groups of antibiotics (1).

Resistance to antimicrobial agents by pathogenic bacteria has emerged in the recent years and is a major health problem. Nanosilver is one of the most commonly used nanomaterials due to its strong disinfectant properties (5); it has a much stronger germicidal capability which can kill up to 650 different types of bacteria (6). The inhibitory effect of ionic silver is due to several biological events such as attachment to cell membranes, alteration of membrane permeability through adsorption to the negatively charged bacterial cell wall, generating reactive oxygen species (ROS), and deactivating cellular enzymes (7). The American Biotech Labs have spent millions of dollars on testing the safety and efficacy of nanosilver technology products. All studies have concluded that nanosilver products are not toxic to cells, animals or humans (8). No toxic effects on human's tissues were reported by low concentration of nanosilver solutions (9).

## 2. Objectives

The purpose of this study was to examine the antibacterial activity of silver nanoparticles and compare them with some antibiotics against clinically isolated *A. baumannii*.

## 3. Materials and Methods

60 bacterial strains were isolated from clinical specimens. *A. baumannii* were isolated from wound, trachea, blood, sputum, pleura, urine and CSF from 8 hospitals in the Tehran province of Iran. All strains were isolated on the usual culture medium, Mueller-Hinton agar (Pro-nadisa Co. Italy), and after an overnight incubation at 35°C, the differentiated test was used to detect the *A. baumannii* bacteria. The oxidative activity of isolated specimens was examined with biochemical by oxidase disk (Rosco Diagnostica A/S, Denmark). Biochemical capabilities of this organism were tested on TSI (Triple sugar iron agar) and LD (Lysine decarboxylase) media. The isolates were classified as *A. baumannii* species based on oxidative negative, non-fermenter and non-motile. *A. baumannii* produces colonies with a 2-3mm diameter at 18-24 hours of incubation. *A. baumannii* did not produce acid from mannitol, sucrose and H<sub>2</sub>S on TSI media and had no reaction on lysine decarboxylase medium (2, 10).

Antimicrobial Effects of Different Antibiotics on *A. baumannii* Bacteria: The susceptibility test against 18 different antibiotics was investigated by the disk diffusion method according to the CLSI, 2010 guidelines (11), used as the reference method for antimicrobial susceptibility testing. Used Antibiotics: Amikacin, gentamycin,

meropenem, piperacillin/tazobactam, gatifloxacin, levofloxacin, cotrimoxazole, nitrofurantoin, ciprofloxacin, imipenem, cefotaxime, ceftazidime, ceftizoxime, clavulanate, ceftriaxone, piperacillin, cefepime and cefixime. The Antimicrobial Effects of Nanosilver on *A. baumannii*: For preparation of nanosilver suspension; firstly silver nitrate was placed under the reducing effect of polyaldehyde, and thus Ag<sup>+</sup> changed to metallic Ag after receiving an electron. Silver particles tend to accumulate together and to prevent this, an acrylic polymer was used in the presence of the surface water. The effect of this surface activator reduced the silver particles' tendency to stick. So a solution with silver particles in the nanometer size was obtained. To confirm the particle size, intensity test was performed on the solution. The average size of silver particles was 6-34 diameters.

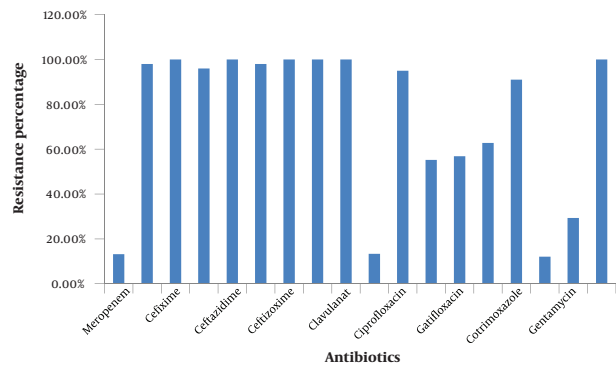
MIC was defined as the lowest concentration of nanosilver preparation that prevented bacterial growth. MICs were determined for all strains by the CLSI reference method (11). Concentrations tested were serial twofold dilutions: 1 (3500 ppm), 1/2 (1750 ppm), 1/4 (875 ppm), 1/8 (437.5 ppm), 1/16 (218.75 ppm), 1/32 (109.37 ppm), 1/64 (54.68 ppm), 1/128 (27.34 ppm), 1/256 (13.67 ppm), 1/512 (6.83 ppm), 1/1024 (3.41 ppm). Disk diffusion tests were performed along with MIC determinations using one inoculum preparation. The MBCs were determined according to the methods outlined by the CLSI (11). The MBC was defined as the lowest concentration of compound that produced a  $\geq 99.9\%$  killing ( $\geq 3\text{-log}_{10}$ ) drop in CFU/ml compared to that of the starting inoculum. Two control samples were used to judge the validity of our work, one of them was free of nanosilver, and the other one was free from bacteria.

## 4. Results

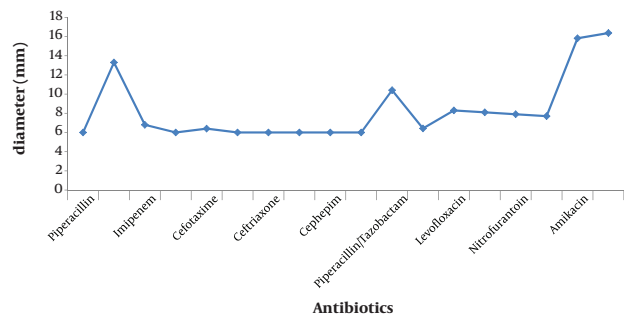
We examined the *A. baumannii* growth in culture media in the presence of nanosilver compared to when the culture media contained different antibiotics; we found that nanosilver dilutions readily prevented growth of the multidrug resistant *A. baumannii*. The information about the susceptibility and resistance rate of the isolates against the 18 different antibiotics used is shown in Table 1 and Figures 1, 2, 3, 4. This information shows the 100% resistance of *A. baumannii* against different antibiotics such as piperacillin, cefixime, ceftazidime, ceftizoxime, cephepim and clavulanat, more than 90% resistance against imipenem, cefotaxime, ceftriaxone, ciprofloxacin and cotrimoxazole, and lower resistance against nitrofurantoin (62.8%), gatifloxacin (56.85%), levofloxacin (55.18%), gentamycin (29.32%), piperacillin/tazobactam (13.34%), meropenem (13.16%), and amikacin (12.07%). Nanosilver examinations showed that the MIC of nanosilver was 27.34 ppm (with average inhibition zone being 8.02 mm), and the MBC was 54.68 ppm (with average inhibition zone being 8.85 mm).

**Table 1.** The Susceptibility of *A. baumannii* Isolates Against Different Antibiotics

	Susceptibility (%)	Average (%)
<b>Effects on Bacterial Cell Walls</b>		
<b>β-lactams</b>	-	16.40
<b>Penicillin</b>	-	-
<b>Piperacillin</b>	0	-
<b>Carbapenems</b>	-	-
<b>Meropenem</b>	86.84	-
<b>Imipenem</b>	1.5	-
<b>Cephalosporins</b>	-	-
<b>Cefixime</b>	0	-
<b>Cefotaxime</b>	3.70	-
<b>Ceftazidime</b>	0	-
<b>Ceftriaxone</b>	1.72	-
<b>Ceftizoxime</b>	0	-
<b>Cephepim</b>	0	-
<b>β-lactamas inhibitors</b>		
<b>Clavulanat</b>	0	-
<b>Combinations</b>		
<b>Piperacillin/Tazobactam</b>	86.66	-
<b>Effects on Bacterial Nucleic Acid</b>		
<b>DNA replication inhibitors</b>	-	27.88
<b>Fluoro quinolones</b>	-	-
<b>Ciprofloxacin</b>	5.17	-
<b>Levofloxacin</b>	44.82	-
<b>Gatifloxacin</b>	43.15	-
<b>Anaerobic DNA inhibitors</b>		
<b>Nitrofurans- derivatives</b>	-	-
<b>Nitrofurantoin</b>	37.20	-
<b>DNA &amp; RNA Synthesis Inhibitors</b>		
<b>Sulfonamides</b>	-	-
<b>Cotrimoxazole</b>	9.09	-
<b>Effects on Bacterial Protein Synthesis</b>		
<b>Aminoglycosides</b>	-	79.30
<b>30 S Protein</b>	-	-
<b>Amikacin</b>	87.93	-
<b>Gentamycin</b>	70.68	-

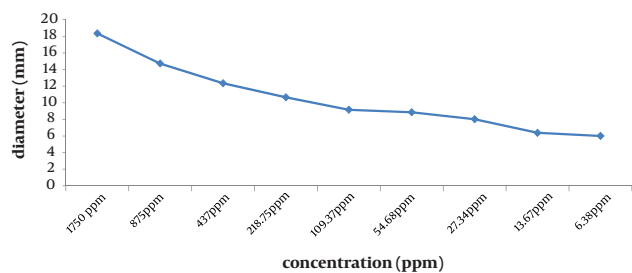


**Figure 1.** Resistance Percentage of *A. baumannii* to the Examined Antibiotics



**Figure 2.** The Average Growth Inhibition Zone of Antibiotics

Susceptibility to nanosilver depended on the concentration. All isolates were susceptible to more than MIC of nanosilver compared to a high resistance to multiple classes of antibiotics. The Average of inhibition zone diameters of different concentrations of nanosilver dilutions is shown in Figure 3. By reducing the concentration of nanosilver, its effectiveness was reduced; at about the 1/128 concentration and lower, inhibition zone of bacterial growth was not observed.



**Figure 3.** The Growth Inhibition Zone Average for Various Nanosilver Dilutions



Figure 4. Antibacterial Disk Diffusion Test for Antibiotics

## 5. Discussion

The rising trend of resistance to antibiotics alarms about the dilemma that could be faced when treating such infections. The results showed that clinical isolates of *A. baumannii* in our hospitals are highly resistant to multiple classes of antibiotics. *A. baumannii* are important opportunistic pathogens with increasing rates of multi-antibiotic resistance due to both intrinsic and acquired mechanisms. In this study we collected 60 samples from different departments of eight university hospitals of Tehran. Several common antibiotics against *A. baumannii* were used for *in vitro* susceptibility test.

This experiment revealed high resistance rates of *A. baumannii*, which were as follows: 100% to piperacillin, cefixime, ceftazidime, ceftizoxime, cephempim, and clavulanat, more than 90% resistance to imipenem, cefotaxime, ceftriaxone, ciprofloxacin, and cotrimoxazole, more than 50% resistance to levofloxacin and gatifloxacin, low resistance to amikacin, meropenem, piperacillin/tazobactam and gentamycin. Other researches in Iran have also shown the high multidrug resistance rates of *A. baumannii* (12, 13). Other studies on *A. baumannii* have shown similar results with respect to these antibiotics (14-16). The resistance percentages reported by Gaur (17) against several antibiotics were different from ours including: ciprofloxacin (81%), cefotaxime (79%), ceftazidime (77.6%), amikacin (74.8%), imipenem (9.1%) and meropenem (9.8%).

Other articles with different results include; the ghulam study (18), imipenem and meropenem (70%), amikacin (52.5%), ceftazidime, cefepime and ciprofloxacin (89%). moniri (19) showed 60% resistance against amikacin, ceftazidim, and ciprofloxacin. There is a common significance to all these articles and that is their alarming results about the high resistance of *A. baumannii* against many used antibiotics. For this reason we decided to find a safe, effective and accessible material with strong effects

on pathogenic microorganism. Nanosilver colloid was made with chemical methods. Size dependent antimicrobial activity of silver nanoparticles has been reported for Gram-negative bacteria (20-22). Small nanoparticles with a large surface area to volume ratio provide a more efficient means for antibacterial activity even at very low concentrations. Silver nanoparticles (Diameter 5-32 nm) enhance the antibacterial activity of various antibiotics (23).

Silver nanoparticles anchor and penetrate the cell wall of Gram-negative bacteria (21, 24). The resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane, and ultimately cell death. It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals, and subsequent free radical-induced membrane damage (6). Ions move into the cells and lead to the production of reactive oxygen species. Based on the greater tendency of silver ions to strongly interact with thiol groups of vital enzymes and phosphorus-containing bases (25) and with the presence of silver nanoparticles inside the cells (21), it is likely that further damage could be caused by interactions with compounds such as the DNA.

This interaction may prevent cell division and DNA replication, and ultimately lead to cell death. In this study we examined the nanosilver efficacy against *A. baumannii* and its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Our results showed a 100% influence by the prepared nanosilver and 27.34ppm MIC and 54.68ppm MBC. Numerous researches have been performed studying the effect of nanosilver on pathogenic microorganism, but there has not been any investigation on the effect of nanosilver on *A. baumannii*, and comparing its effects with different classes of antibiotics. Our results were quiet similar to the Humberto (26) study on *Pseudomonas aeruginosa*.

The MIC calculation for another gram negative bacteria, *Escherichia coli*, was reported later by Amany (27) and Petrus (28), which was similar to our results. In conclusion silver nanoparticle is a strong bactericide agent to the multidrug resistant *A. baumannii* strains, which is an important cause of hospital acquired infections. Thus, preparing new bactericidal agents in combination with nanosilver particles can be a useful route for introducing new antibiotics to prevent a variety of nosocomial infections. Finally, nanosilver products can be used for an effective prevention and treatment of some infectious diseases.

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## Authors' Contribution

None Declared.

## Financial Disclosure

None Declared.

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