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GAVIN PUBLISHERS

International Journal of Bacteriology & Parasitology

Karimipour SN, et al. Int J Bacteriol Parasitol: IJBP-106.

DOI: 10.29011/ IJBP-106. 000006

Research Article

Evaluation of Antibacterial Activity of Nano silver Particles on Clinical Isolates of *Acinetobacter baumannii*

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Citation: Karimipour SN, Tanomand A, Hosainzadegan H, Zadeh AH (2018) Evaluation of Antibacterial Activity of Nano silver Particles on Clinical Isolates of *Acinetobacter baumannii*. Int J Bacteriol Parasitol: IJBP-106. DOI: 10.29011/ IJBP-106. 000006

Received Date: 07 March, 2018; **Accepted Date:** 22 March, 2018; **Published Date:** 02 April, 2018

Abstract

Acinetobacter baumannii is a gram negative and obligate aerobe bacillus, and an opportunist pathogen that causes hospital acquired infections. Treatment of related infections of this bacterium is difficult because of extensive antibiotic resistance. Considering this problem, the effectiveness of different antimicrobial drugs has been studied to combat infections caused by this bacterium. The aim of this study is to evaluate the effectiveness of Nano Silver particles on clinical isolates of *A. baumannii*.

Twenty clinical strains of *A. baumannii* and *A. baumannii* reference strain NCTC 12516 were obtained from Imam Reza Hospital of Iran-Tabriz. Antimicrobial activity of two types of Nano Silver particles were evaluated on mentioned clinical and reference bacteria. One of the nanoparticles was purchased from Pishtazan Nanotechnology Corporation (Iran-Mashhad), and the other was gifted by chemistry department of Maragheh University, with 20, 5 nm diameters, respectively. Concentration of nanoparticle solutions was confirmed by spectrometry. Then MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of the Nano Silver solutions were estimated by serial dilutions in nutrient broth and 0.5 McFarland turbidity under standard methods. Sensitivity of strains were evaluated by disk and well diffusion.

Based on the experiments 1250 to 2500 ppm, and 156 to 312 ppm (of 20 and 5 nm Nano Silver particles respectively) were determined as MIC and MBC on studied bacteria. MIC and MBC of clinical *A. baumannii* strains had no significant difference with the reference strain. In the disk and well diffusion assays, growth inhibition of 20000 ppm of 20nm Nano Silver solution for both clinical and standard strains were 11 and 9.5 mm, respectively. *A. baumannii* is an innate resistant bacterium, but its sensitivity to Nano Silver, and the similar MIC and MBC values of different clinical strains indicates that there is no resistance against Nano Silver particles. So, Nano silver materials could be used as a safe antimicrobial against infections of this kind of bacteria.

Keywords: *A. baumannii*; Minimum Bactericidal Concentration; Minimum Inhibitory Concentration; Nano silver

Introduction

Recently nanotechnology has produced developments in research and materials [1]. Based on this technology new products have been produced in nanometer dimensions [2]. Usually nanoparticles have measurements from 1-100 nanometers. Decreasing the size of nanoparticles affects their properties as nanoparticles have optical, physicochemical and biological characteristics based on their sizes [3] thus changing the size of nanoparticles could improve their functions [4]. Nano Silver is a nanoparticle associated with an ancient history of consumption of silver by human beings. Recently it has been understood that Nano Silver has very potent antimicrobial activity than silver itself [5,6]. Results of different studies have been indicated that

Nano Silver particles acting as wide spectrum agent on all kinds of microbes including bacteria, fungi, protozoa, and viruses, while antibiotics acting only on the bacteria [7,8]. Interactions between nanoparticles and macromolecules of organisms have been evaluated in multiple studies. Negative charge on microorganisms acts as an electromagnetic with positive charge on nanoparticles, and absorbs nanoparticle on the cells, which in turn could kill microbial cells. Finally absorbing high amounts of nanoparticles, oxidizes surface molecules of microbes and kills them faster. Probably ions releasing from nanoparticles, have interacting with thiol (SH-) groups of surface proteins of bacteria. Some of these cell membrane proteins having role in the transferring of minerals from cell wall, which inactivating of them and leading to impermeability of membrane [9], which leads to cell death. Nanoparticles also delaying the adhesion and biofilm formation of bacteria, which causes to some bacteria couldn't fix and multiply locally [10]. Antimicrobial changes leading to growth inhibition of pathogenic bacteria are a favorite aim of antimicrobials research. Colony formation, growth and formation of compressed matrix biofilms have make bacteria resistant against defense mechanisms of hosts. Nanoparticles inhibiting of mentioned activities by bacteria and making them vulnerable to immune system. Nanomaterials made of metals having extended bactericidal, fungicidal, and virucidal effects. Nanomaterials destroying enzymes and DNA of microorganisms because of having surface charge and ratio of surface to volume [11,12].

Materials and Methods

Nano Silver Preparation: Two kinds of Nano Silvers were used in this study. One with 20 nm diameter have been purchased from Pishtazan Nanotechnology Corporation (Iran-Mashhad), and the other with 5 nm diameter was synthesized and gifted by the Department of Chemistry (Dr. Rostamnia of Maragheh University).

Materials and Bacteria: Clinical isolates of *A. baumannii* and standard NCTC 12516 were prepared from Imam Reza Hospital, Iran-Tabriz. Biochemical tests including gram reaction, Catalase, oxidase and PCR have been used for identification of isolates. Muller- Hinton agar and Nutrient broth have been used for well and disk -diffusion, and serial dilutions.

Study of Antimicrobial activity of Nano Silver Particles

Determination of MIC and MBC: 0.08 g of each of the Nano Silvers [20 and 5 nm] was resolved in 2 ml and a serial dilution was prepared in nutrient broth. 10 ml of 0.5 McFarland turbidity (1.5×10^8) *A. baumannii* suspensions was added to each of the serial diluted tubes. So different concentrations of Nano Silver were shaker incubated with constant number of bacteria for 24h and 37°*C*. Then 10 µl of cultures was diluted with 15 ml of PBS, and cultured in Muller-Hinton agar for colony counting. Plates were incubated for 24h at 37°*C*. Finally, *the growth of bacteria was evaluated and MIC and MBC of* Nano Silver*s was determined based on growth of bacteria.* The concentration of Nano Silver which killed 99.99% of bacteria was considered as MBC, and cultures with no growth were considered as MIC concentration.

Assessment of antimicrobial activity of Nano Silvers by well-diffusion and Disk-diffusion: Clinical *A. baumannii* isolates were cultured in nutrient broth tubes, pellets were collected by centrifugation. 0.5 McFarland dilutions were prepared of strains. Then a standard lawn culture was prepared with sterile swabs on Muller-Hinton agar. Different concentrations of 20nm (5000,10000,20000 and 40000ppm), and 5 nm Nano Silver (625,1250,2500 and 5000 ppm) were point inoculated on agar media with a minimum of 15mm of distance from each other. Plates were incubated for 24h at 37°*C*, after that growth inhibition zone was measured with ruler. In disk-diffusion four blank disks were put on the lawn cultures ascetically, and then 30 µl of each of the dilutions of Nano Silvers were added to the disks. Finally, after incubation for 24h at 37°*C, growth inhibition zone was measured with ruler.* All experiments were repeated three times and the results were calculated as mean of 'repeats'.

Results

MIC and MBC evaluations of clinical *A. baumannii* isolates in nutrient broth was estimated 1250 and 2500 ppm, 156 and 312 ppm for 20nm and 5 nm Nano Silvers, respectively. MIC and MBC were determined by tube dilution as indicated in (Figure 1).

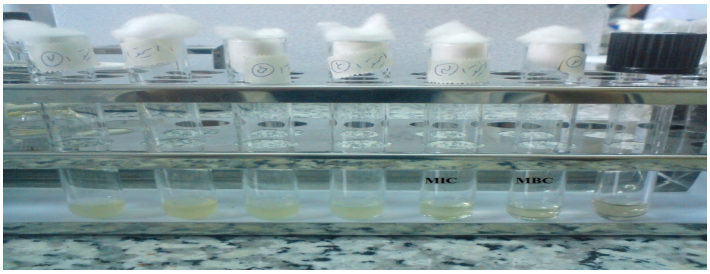

Figure 1: A sample of tube dilution and growth inhibition of 20 and 5 nm Nano Silvers on *A. baumannii*.

Results of well-diffusion indicated that inhibition zone diameter increased with increasing Nano Silver concentration. Variations of means of inhibition zone diameter of 20 and 5nm nanoparticles on *A. baumannii* isolates are shown in Diagram 1.

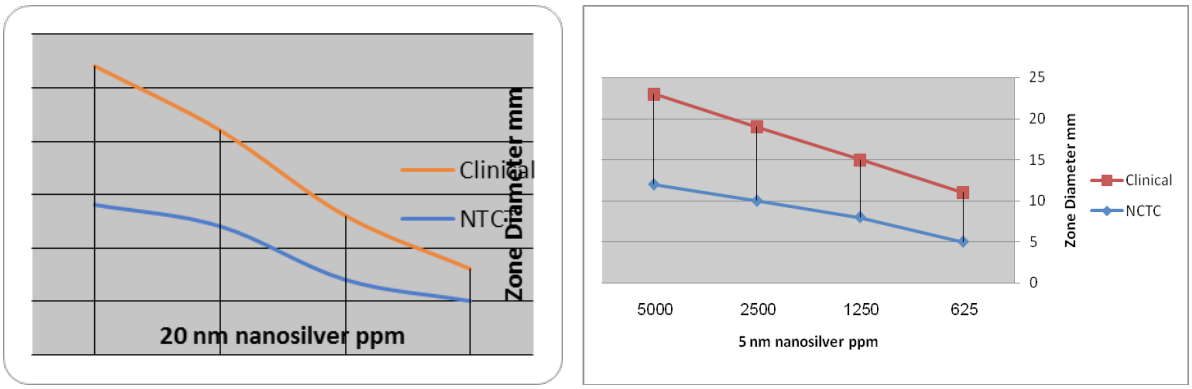

Diagram 1: Means of inhibition zone diameters of different concentrations of 20 and 5nm Nano Silvers on *A. baumannii* isolates and reference strain in well-diffusion.

Results of Disk-diffusion were similar to well-diffusion and zone diameters have been increasing with increasing Nano Silver concentration. Variations of means inhibition zone diameter of 20 and 5nm Nano Silvers on *A. baumannii* and reference strains have been indicated in Diagram 2.

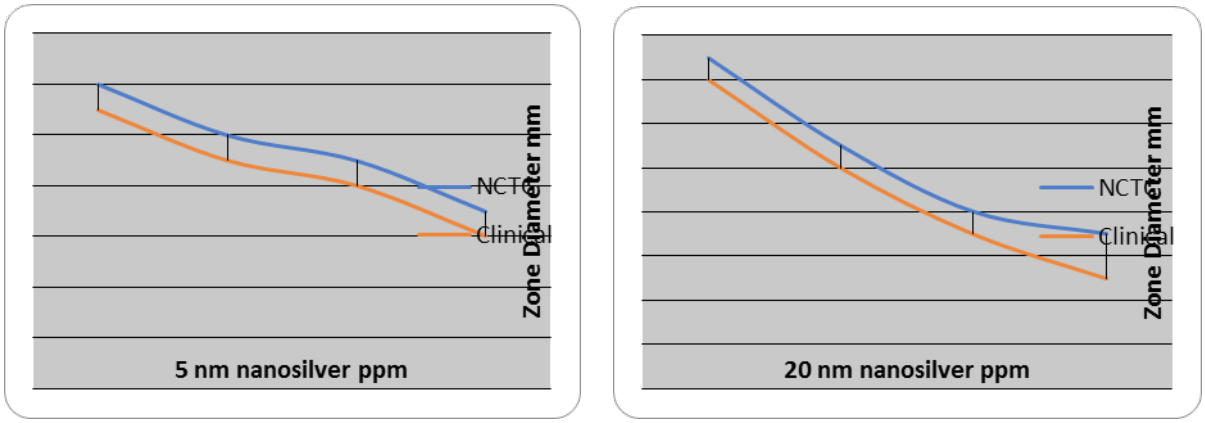

Diagram 2: Inhibition zone diameter of different concentrations of 20 and 5 nm Nano Silvers on *A. baumannii* isolates and reference strain in disk-diffusion.

Results of antimicrobial effects of 20 and 5 nm nanoparticles with well and disk diffusion methods have been indicated in figures 2 and 3 as follow.

20 nm nanosilver

Figure 2: Disk and well diffussion of 20nm Nano silvers on *A. baumannii* and reference strains. 1,2 and 3,4 are referring to *A. baumannii* and reference strains in disk and well diffusion tests respectively.

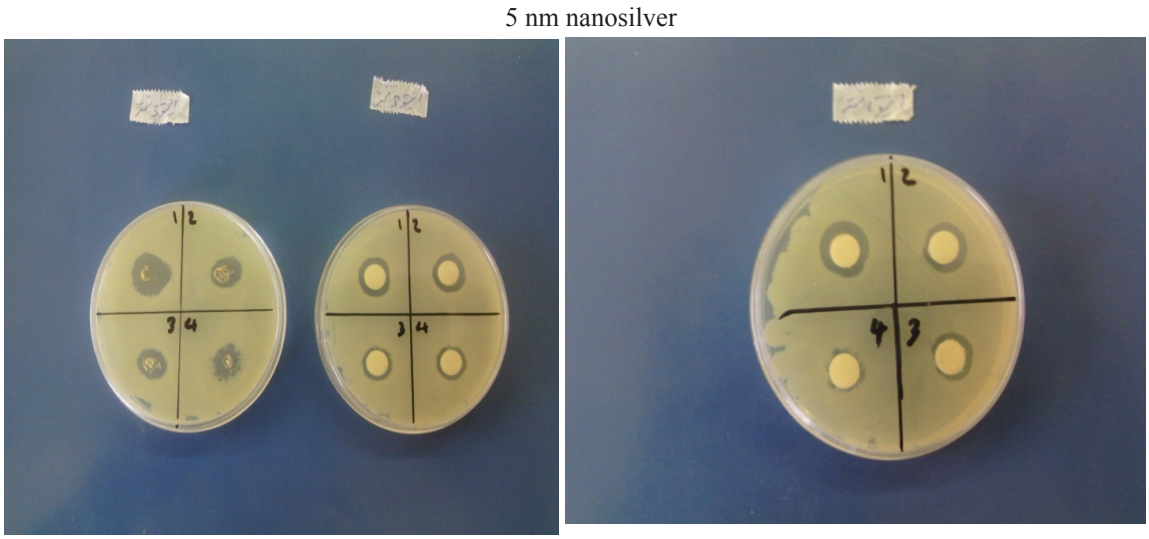

Figure 3: Well and disk diffusion of 5nm Nano silvers on *A. baumannii* and reference strains.1,2 and 3,4 are referring to *A. baumannii* and reference strains in well and disk diffusion tests respectively.

Discussion

A. baumannii is a gram negative non-motile coccobacillus, which rarely causes severe infections in immunocompetent individuals. This bacterium is not known as natural microbiota of human body. Different strains are mainly resistant to antibiotics that could be innate or acquired by genetic factors. Carbapenem resistant strains specially in hospital infections making a serious challenge in hospital settings. A few studies have shown that isolated clinical strains from Iran are extensively resistant to many classes of antibiotics, and Nano Silver acts successfully on multi-resistant bacteria [13,14]. In Nasir's and colleagues study on 20 *P. aeruginosa* clinical isolates, it is reported that MIC on Nano Silver was mainly in 100 ppm concentrations, and only 6 strains were inhibited by it [14]. Using Nano Silver is one of the effective methods of combating with antibiotic resistant bacterial infections, especially in combination with antibiotics Nano Silver seems potentiates the survival rate of mouse models with peritonitis of *A. baumannii* in comparison with standard drug treatments [15].

In this study antibacterial effect of different size Nano Silvers were evaluated on clinical *A. baumannii* isolates. Tube broth dilutions of Nano Silver indicate that in low concentrations it can inhibit the growth of *A. baumannii*, which is in accordance with Sondi's, et al. study on *E. coli*. Sondi, et al. showed that Nano Silver particles accumulating on the bacterium, and some of it penetrating into the cell. Differences in distribution of Nano Silver particles could be relating to the size of particles [16]. Our results in disk and well-diffusion methods indicate that increasing concentrations of Nano Silver have increases the zone inhibition diameter on *A. baumannii* isolates. Zone inhibition diameter have complete correlation with Nano Silver dose, which are in accordance with similar results of Nano Silver effects on *S. aureus* and *E. coli* by Kim, et al. [7]. Niakan, et al. in their study determined the MIC and MBC of 6-34 nm diameter Nano Silvers against drug resistant *A. baumannii* isolates, which was similar to our study. Means of MIC and MBC values were estimated from 27.34 ppm to 54.68 ppm, respectively, and all of the isolates (100%) were susceptible to concentrations more than MIC of Nano Silver [13]. In a study Alizadeh et al found that *Brucella melitensis* strains were sensitive to Nano Silver, in vitro and animal models. Antibacterial activity was dependent on concentration of nanoparticles [17], which also observed in this study. Humberto, et al. in a study have confirmed the bacteriostatic effects of Nano Silver on the antibiotic resistant bacteria (including *P. aeruginosa*, ampicillin-resistant *E. coli*, and erythromycin-resistant *S. pyogenes*). As we know *P. aeruginosa* has carries many of plasmid and chromosomal antibiotic resistant factors that makes difficult the treatment of related infections, so that new antibiotics have couldn't decrease mortality of infections [18]. Morones, et al. found that Nano Silver have antibacterial effects on different kinds of gram negative bacteria, they found that Nano Silver attaches to cell wall surface, and after releasing silver ion, destroys permeability of cell membrane [19]. Effects of nanoparticles size on antibacterial activity have been studied by many researchers, and Baker, et al. indicated that decreasing nanoparticles sizes increases the surface to volume ratio, and in turn increases antibacterial activity of nanoparticles [20].

Finally, our study indicates that Nano Silver have potent antimicrobial activity against antibiotic resistant *A. baumannii* isolates. On the other hand, there weren't reports of resistance against Nano Silvers. So, Nano Silver has been proposed as a safe, cheap and friendly-use antimicrobial in resistance cases of bacterial infections. Although its toxicity should be examined for further judgments for clinical use.

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