Int J Basic Sci Med. 2016;1(1):25-28 Original Article

http://ijbsm.zbmu.ac.ir/ doi 10.15171/ijbsm.2016.06

Evaluation of the Antimicrobial Activity of Silver Nanoparticles on Antibiotic-Resistant *Pseudomonas aeruginosa*

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Published online 29 June 2016

Please cite this article as follows: Nasiri A, Afsar Gharebagh R, Nojoumi SA, et al. Evaluation of the antimicrobial activity of silver nanoparticles on antibiotic-resistant Pseudomonas aeruginosa. Int J Basic Sci Med. 2016;1(1):25-28. doi:10.15171/ijbms.2016.06.

Abstract

Background: Antimicrobial resistance is one of the major characteristics of infectious agents. Silver nanoparticles (AgNPs) have been introduced as novel antibacterial agents in accordance with the traditional treatments. Our purpose of this study was to evaluate the antimicrobial activity of AgNPs on the *Pseudomonas aeruginosa (P. aeruginosa)* that are resistant to antibiotics.
Methods: During a cross-sectional study, we tried to evaluate 20 strains of *P. aeruginosa* isolated from the urine cultures of patients admitted to the hospital due to urinary tract infections. The AgNPs were commercially purchased. The minimum inhibitory concentration (MIC) of AgNPs in different concentrations was determined by the dilution in wells on bacteria. The antibiotic susceptibility pattern of *P. aeruginosa* was evaluated by the Kirby-Bauer disk diffusion standard.
Results: Current study indicated that *P. aeruginosa* were resistant to four types of agents including ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), and ciprofloxacin (15%) and result of nanosilver indicated that the most MIC was 100 ppm concentration, and six strains of *P. aeruginosa* were inhibited by it.
Conclusion: Our study presented a new type of silver nanoparticle and indicated that they can be embedded in bone cement to prevent infections once synthetic conditions are tailored for such applications.

Keywords: Nanosilver, Antimicrobial activity, *Pseudomonas aeruginosa*, Minimum inhibitory concentrations (MIC), Antibiotic-resistant

Introduction

In recent decades, several studies have been carried out regarding the transport of materials, particularly precious metal particles in the nano regime. Silver nanoparticles (AgNPs) show special properties that are great scientific achievements in nanotechnology. Their use is widespread as they are produced in different ways depending on the type of material and its applications. Nanoparticles are among the most common elements in science and nanotechnology. They hold interesting properties; in this regard, they have a wide variety of applications in the chemical, medical, and pharmaceutical industries, as well as electronics and agriculture. In their chemical composition, these particles are composed of metal, ceramic, polymer, and semiconductor. Metals such as silver are considered safe and are effective antibacterial agents that can kill more than 650 types of microorganisms including bacteria and virus. By increasing the surface to volume ratio, the nanoscale silver antibacterial properties can be improved.[1] AgNPs are produced usually based on optical methods,[2,3] restoring either the chemical[4] or silver oxide.[5] Like other technologies, nanotechnology can be followed by several disadvantages; for example, experts do not have enough information in this case, and whether the destructive effects of AgNPs pertain to cells and tissues. It is possible that nanoparticles may have serious consequences for the environment and human health.[6] *P. aeruginosa* is a gram-negative bacterium that is usually seen alone or in short chains. Colonies of these bacteria are smooth and rounded, often in green fluorescent dye that is used to identify these bacteria. In the regimens, a drug is not used alone and penicillin for example is usually used in combination with an amino glycoside like gentamicin.[7] The aim of this study was to investigate the antimicrobial effect of AgNPs against *P. aeruginosa* that are resistant to antibiotics.

Methods

Bacterial Strains

All 20 strains of *P. aeruginosa* were isolated from the urine cultures of hospitalized patients with urinary tract infections**.** The cultures were grown on nutrient agar. To identify different kinds of pseudomonas from tests, gram staining, catalase, oxidize, glucose tests, OF (oxidation fermentation), and TS (triple sugar iron) were used.[8] *P. aeruginosa* were detected from urine specimens, transferred to microbiology laboratory, using Gram-Negative Identification Panel Type II (Dade International Inc., West Sacramento, Calif.). Microdilution broth testing (MicroScan; Dade International Inc.) was applied to test the susceptibility.

Agar disk diffusion assay

Susceptibility of all antibiotics was assessed using the disc diffusion method on the Muller-Hinton agar recommended by the Clinical and Laboratory Standards Institute (CLSI).[9] *Staphylococcus aureus (S. aureus)* isolated plates were grown overnight on the blood agar and the colony suspension was prepared using sterile saline water equivalent to a 0.5 McFarland standard. The suspension (100 µL) was spread over media plate and antibiotic disc was transferred on the aseptic surface of this inoculated plate. The isolated plates were then tested with different antibiotics viz ampicillin, nitrofurantoin, nalidixic acid, and ciprofloxacin, and their concentrations were illustrated in parenthesis.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Nanosilver

AgNPs powder used in this study was manufactured by Thermolon Korea Inc., according to broth microdilution method. Then the serial doubling dilutions of AgNPs were prepared in a 96-well µL plate ranged from 12.5 ppm to 200 ppm. Ten microlitter of the indicator solution and 10 µL of the Mueller Hinton Broth were added to each well. Finally, 10 µL of the bacterial suspension (10^6 CFU/mL) was added to each well to obtain a concentration of 10^4 CFU/mL. Plates were wrapped loosely with clingfilm to ensure that bacteria did not get dehydrated. Moreover, plates were prepared in triplicates and were placed in an incubator at 37°C for 18-24 hours. Color change was then assessed visually. The lowest concentration at which color change occurred was taken as minimum inhibitory concentration (MIC) value. MIC is defined as the lowest concentration of extract at which microorganism does not demonstrate visible growth. Microorganism growth was indicated by turbidity. Minimum bactericidal concentration (MBC) was defined as the lowest concentration of nanosilver at which the incubated microorganism was completely killed. Microorganism growth was indicated by turbidity and *P. aeruginosa* ATCC27853 was the positive control in this study.

Statistical Analysis

Results were described as mean or frequency. Comparing the mean values among groups was performed using one-way analysis of variance. All data analyses were conducted using SPSS version 19.0 software. *P* value less than 0.05 was considered statistically significant.

Results

The average size of AgNPs was 20 nm. The results showed that isolated pseudomonas were respectively resistant to antibiotics; however, overall *P. aeruginosa* were resistant to four types of the agents including ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), and ciprofloxacin (15%) (Table 1). The result of nanosilver showed that the most MIC was 100 ppm concentration, and six strains of pseudomonas were inhibited (Table 2), and the least MIC was 12.5 ppm concentration, and one strain of Pseudomonas was inhibited. We also found that the antifungal effects were increased by increasing the concentration of the nanosilver. No significant difference was observed between the Inhibition of bacterial growth, regarding the nanosilver concentration ($P < 0.05$).

Discussion

Since a long time ago, scientists have detected the antimi-

Table 1. Percentage of Antimicrobial Susceptibility of 20 Strains of *P. aeruginosa*

	AM	Fm	NA	CP
S	0	60	10	60
I	15	20	25	25
R	85	20	65	15

Abbreviations: CP, ciprofloxacin; NA, nalidixic acid; Fm, nitrofurantoin; AM, ampicillin; S, sensitive; I, intermediate; R, resistant.

Table 2. MIC and MBC of Nanosilver Against *P. aeruginosa*

	3.1	6.25	12.5	25	50	100
MIC	0	0	5	10	55	30
MBC	0	0	0	5	10	70

Abbreviations: MIC, minimum inhibitory concentration; MBC, Minimum bactericidal concentration.

crobial properties of silver.[10] However recently, just due to the form of nanoparticles, their surface area has increased by more than 99% and therefore their antimicrobial properties have increased.[11]

In this study, the size of AgNPs was 20 nm and in 100 ppm nanosilver, the percentage of bacterial reduction was 30%, while in 50 ppm that more nanosilver was used, the percentage decreased to 55%.

Haji Mirzababa et al[12] showed that the nylon carpet was stained with 25 ppm nanosilver and the percentage of bacterial reduction was 72.3 %, but in cases where the floor was covered with 50 ppm that more nanosilver was used, the percentage decreased to 99.99% of bacteria. In addition, checking the stability of AgNPs on samples was standard and 1 to 10 times more stable after washing.

Chahar and Khodaday[13] showed that AgNPs synthesis in the fruit juice shows maximum absorption at 420 nm. Moreover, the antimicrobial activities of AgNPs against *E. coli*, *S. aureus, P. aeruginosa*, and *B. sabtilis* have been proven.

Alizadeh et al[14] showed that even low concentrations of AgNPs can inhibit the *Brucella melitensis*. The MIC and minimum lethal concentration of AgNPs in broth macrodilution were reported as 6 ppm and 4 ppm, respectively. In addition, anti-brucella effects of AgNPs have been observed in a mouse model.

Hernández-Sierra et al[15] demonstrated that AgNPs in comparison to zinc oxide and gold nanoparticles prevent the growth of streptococcus mutants with a lower concentration.

Sintubin et al[16] showed that the lowest concentration of an antimicrobial silver nanoparticle for *Staphylococcus aureus* was 20 mg/mL.

In the present work, *P. aeruginosa* were resistant to four types of agents including ampicillin (85%), nitrofurantoin (65%), nalidixic acid (65%), and ciprofloxacin (15%).

Although large national surveys provide critical information about resistance situations, they do not address the potentiality of much higher rates of resistance within individual communities and hospitals. For example, during 2001 and 2006, rates of nonsusceptibility among the *P. aeruginosa* isolates in Brooklyn, NY ranged from 27% to 29% for cefepime, 30% to 31% for imipenem, 23% for meropenem, and 41% to 44% for ciprofloxacin.[17]

According to the results of the study carried out by Nikokar et al,[18] it was indicated that from 182 patients admitted in burn center, 86 (47%) had *P. aeruginosa* in their isolated samples with the following resistance situations: cloxacillin (91.8%), cotrimoxazole (86%), cephazolin (83.7%), carbenicillin (74.4%), piperacillin (69.9%), ceftazidime (68.8%), ciprofloxacin (66.3%), tobramycin (58.2%), amikacin (48.8%), and gentamicin (37.2%). The least resistance was detected for imipenem as 23.3%. Out of them, 39 (45.3%) isolates were detected as multi-drug resistant. Investigation by PCR reaction showed that 37 (43%) of the *P. aeruginosa* isolates and 27 (69.2%) of the multi-drug resistant strains harbored class 1 integrons. A significant relationship was observed between the presence of integrons and the resistance against imipenem, ceftazidime, piperacillin, and ciprofloxacin ($P<0.001$).

Ahmadi et al[19] reported that *P. aeruginosa* strains had the highest levels of resistance against ampicillin (93%), gentamycin (89.5%), ciprofloxacin (82.5%), and amikacin (77.3%). The most effective drugs were meropenem (2.3%), imipenem (2.9%), polymyxin B (21.5%), and cotrimoxazole (31.9%).

Moazami-Goudarzi and Eftekhar[20] showed 99.2% resistance to carbenicillin, 98.4% to ticarcillin, 96.2% to ciprofloxacin, 95.4% to co-trimoxazole, 94.7% to imipenem and meropenem, 93.9% to piperacillin, 93.2% to azetronam, 92.4% to tobramycin, and 87.2% to piperacillin-tazobactam. Totally, 100% of the isolates showed multidrug-resistance (resistant to more than 3 classes of antibiotics such as imipenem).

Fazeli and Momtaz[21] showed that the most fluoroquinolone resistant bacteria were encoding the gene *gyrA* (15. 68%). They also observed high level of resistance to penicillin (100%), tetracycline (90.19%), streptomycin (64.70%), and erythromycin (43.13%).[21]

Conclusion

Our study provided evidence regarding the antimicrobial characteristics of a new type of silver nanoparticle which can be effective in protection against infections due to different organs.

Ethical Approval

Not applicable.

Competing Interests

Authors declare that they have no competing interests.

Acknowledgements

The authors would like to thank Research Center of Agricultural Biotechnology.

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