J. Water Environ. Nanotechnol., 3(1): 51-57 Winter 2018

ORIGINAL RESEARCH PAPER

Genomic Effect of Silver Nanoparticles in *Staphylococcus aureus* Bacteria

Vida Alizadeh, Bahram Golestani Eimani*, Fariba Amjady

Department of Biology, Urmia Branch, Islamic Azad University, Urmia, Iran

Received: 2017.07.28 Accepted: 2017.10.14 Published: 2018.01.30

ABSTRACT

Background and objectives: Drug resistance in bacteria is one of the important problems in the antibacterial field. Therefore, new drugs and therapeutic approaches are required to eliminate bacteria using different and novel mechanisms. Among these, the silver nanoparticles have been proposed as a substance with antibacterial properties against gram-positive and gram-negative bacteria. The present study aimed to investigate the effects of silver nanoparticles with a size of less than 20 nm on the genome of *Staphylococcus aureus* (*S. aureus*) as a model for gram-positive bacteria.

Material and methods: For this purpose, the bacteria were treated at concentrations of 100 and 150 µg/ml nanoparticles and antimicrobial properties of the nanoparticles were investigated in intervals of 2, 4 and 24 hours, then DNA was extracted. RAPD molecular marker was used to investigate the effects of nanoparticles on the genome. In addition, the results of electrophoresis for polymerase chain reaction (PCR) products on agarose gel were analyzed.

Results: The present findings demonstrated that silver nanoparticles not only have an inhibitory effect on bacteria but also affect the genomic DNA sequence of this bacterium and change it in different sites.

Conclusion: The nanoparticles are antibacterial compounds and can be an appropriate alternative to antibiotics.

Keywords: *Genetic Diversity, Growth Inhibition, NTSYS-PC Software, Random Amplified Polymorphic DNA (RAPD-PCR), Silver Nanoparticles*

How to cite this article

Alizadeh V, Golestani Eimani B, Amjady F. Genomic Effect of Silver Nanoparticles in Staphylococcus aureus Bacteria. J. Water Environ. Nanotechnol., 2018; 3(1): 51-57. DOI: 10.22090/jwent.2018.01.005

INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that can cause human infections as skin ulcers and lethal diseases such as bacteremia, endocarditis, pneumonia, septicemia and osteomyelitis [1]. More than 90% of *S. aureus* have become resistant to antibiotics such as penicillin, methicillin, aminoglycoside, macrolides, and lincosamides [2, 3]. A lot of efforts and investments have been made to develop the drugs and novel therapeutic purposes against different bacterial species [4]. Antibacterial agents are used in the textile industry, water disinfection, medicine, food packaging etc. [5]. In the past few decades, the nanomaterials have attracted more attention due to some unique features like large surface area to volume ratio and high reaction activity [6,7]. The nanoparticles generally are defined as the particles less than 100 nm in size. This small size provides them unique properties. The nanoparticles due to having an extent surface area to material mass have higher reaction activity that is often in associated with a high lethality level [8]. Silver nanoparticles have antibacterial effects against a wide range of drug-resistant bacteria. Lara *et al*. demonstrated strong antibacterial activity for silver nanoparticles

* Corresponding Author Email: *golestani_bahram@yahoo.com*

against bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus pyogenes* that are resistant to ampicillin and erythromycin and became multidrug-resistant [9]. Biofilm formation in bacteria is one of the major unknown problems because of protecting pathogenic bacteria against antibiotics, developing chronic infections [10]. Electrostatic properties of nanoparticles and biofilms affect their interaction [11]. In addition, the silver nanoparticles react with the bacterial cell membrane to destroy. The silver ions interact with essential biological molecules such as sulfur, oxygen and nitrogen, thereby preventing the bacterial growth [12].

The silver nanoparticles are bound with antibiotics such as penicillin G, amoxicillin, erythromycin and vancomycin; this cooperation leads to increase antimicrobial effect against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria [13]. Ag^+ utilizes several antimicrobial mechanisms inside microbial cells (14). Ag^+ inhibits the electron transport chain in microbial cytochrome [15, 16]. Ag^+ is combined with microbial DNA and RNA to cause damage [4, 9, 14,17,18]. Ag^+ inhibits protein translation by destructing 30S ribosomal subunit [9]. And the cell wall synthesis in gram-positive bacteria [9]. Ag^+ ions also form the ROS, which can be fatal for both bacterial cells and eukaryotic host cells [9, 14 and 19]. However, the targeted use of silver nanoparticles with antimicrobial properties requires more knowledge on environmental compatibility. Toxicity and cytotoxicity of antibacterial nanoparticles on bacterial and human cells should be considered simultaneously. Usually, heavy metal ions (Ag^+ and Cu^+) have been recognized as fatal substances. However, their lethal doses for human cells are relatively high. Typically, Ag^+ ions at low concentrations (0.001-0.050 ppm) are effective bactericide [20, 21] so it could also affect other organisms and eukaryotic cells during actions as antibacterial substances.Therefore, there are needs to conduct further studies in order to obtain effective antibacterial concentrations and its genotoxic effect as a suitable alternative for antibiotics and harmless disinfectants.This study was conducted to evaluate the effect of different dosages and duration of treatments with silver nanoparticles on the genome of the *S. aureus* as a bacterial model. For this purpose, RAPD-PCR technique was used in this study.

MATERIALS AND METHODS

In the present study, the *S. aureus* bacterium was cultured on eosin methylene blue (EMB) and blood agar media and then was passaged to 5 ml of brain heart broth (BHB) broth in order to assess the inhibitory properties of nanoparticles and DNA extraction.

Assessment of antimicrobial properties of silver nanoparticles

silver nanoparticles less than 20 nm in diameter were synthesized by the Nanotechnology South Korea. Phosphate-buffered saline (pH=7.4) was applied as a solvent for silver nanoparticles in order to prepare a stock solution for silver nanoparticles.

To investigate the effects of nanoparticles on bacteria, the concentrations of 100 and 150µg/ml of silver nanoparticles were inoculated into each test tube. The test tubes were placed in the shaking incubator at 37 °C overnight with a speed of 200 rpm and the optical density of each test tubes was measured at a wavelength of 600 nm during intervals of 2, 4 and 24 hours in order to evaluate bacterial growth.Before treatment, the test tubes without nanoparticles were used as a control as above method.

DNA extraction and RAPD-PCR

DNA extraction of treatment and control bacteria was carried out using a DNA extraction kit based on the instruction (Cinnagen Co., Iran). Its quality and quantity were analyzed on 1% agarose gel electrophoresis and spectrophotometry.

Eleven 10-nucleotide random primers (Cinnagen Co., Iran) were used to examine genomes of control and treated bacteria using RAPD-PCR. Primers sequences and properties are given in Table 1. Each component of the polymerase chain reaction to perform RAPD-PCR techniques included 1 ml of extracted DNA samples, 2.5 µl (x10) of PCR buffer, 3 µl of $MgCl_2$, 1 µl of dNTP mix, 1µl of primers and 0.3 µl of DNA Taq polymerase. The volume of PCR reaction solution was brought to the volume of 25 µl with deionized distilled water. PCR reaction was carried out in Thermal Cycler device (Corbett Research, Australia) under a program, including five-minute cycle at 95 °C for initial denaturation of DNA template and then 40 cycles involving denaturation of DNA template strands at 95 °C for 35 seconds, primer annealing at 30 °C for 45 seconds, the extension phase at 72 °C for 45 seconds and finally a 7 minute cycle at 72 °C to

Table 1: Results of banding related to primers of Fig. 2

Primers	band	C	T1	T2
OPB	1100	1	1	1
	>1500	1	0	1
OPD	1350	1	1	1
	450	1	1	1
	350	0	1	0
	1550	0	1	1
	1500	0	1	1
OPS	1100	1	1	1
	800	0	0	1
	400	1	1	1
	>1500	0	1	0
	1100	0	1	0
OPQ17	1000	0	1	1
	800	1	1	0
	250	0	1	0
	1100	0	1	1
OPS.5	950	1	1	1
	640	0	1	1
	>1500	0	1	0
	1500	0	1	1
	1300	0	1	1
	1100	0	1	1
OPA11	900	1	1	1
	800	1	1	1
	700	0	1	0
	600	1	1	0
	400	1	0	1

complete the final extension. After optimizing the PCR conditions, the combinations and the thermal profile were used for all 11 primers.

The proliferated products on 2% agarose gel containing red safe were electrophoresed in the TBE buffer (1x) for 5 hours with a voltage of 100 V. DNA ladder marker with a size of 100-bp was used to determine the size of the product and was imaged by Gel Doc (Uvitec, France). Scoring the bands obtained from RAPD analysis was conducted according to the presence of bond (1) and the absence of it (0); the data are given in Tables 1 to 3. Then the data were inserted in software based on the molecular weight. The similarity matrix was calculated via DIC method and dendrogram was drawn using the UPGMA method in NTSYS-PC software.

RESULTS

Assessment of antimicrobial properties of silver nanoparticles

The results of evaluating of antibacterial effects of silver nanoparticles at different concentrations and times on *S. aureus* are given in Figs. 1. Adding silver nanoparticles to the culture medium of bacteria after 2, 4 and 24 hours caused significant changes in the reduction in bacterial growth at the concentrations of 100 and 150 µg/ml, proving the effectiveness of these particles as suitable antimicrobial compounds.

Analysis of RAPD- PCR products

Electrophoretic bands obtained from amplification of 11 primers by RAPD- PCR have been presented in Figs. 2 and 3. In each figure, primers from left to right are respectively related to the control sample, first treated sample with the concentration of 100 µg/ml and the second treated sample with the concentration of 150 µg/ml.

Then the sizes of the bands obtained from RAPD

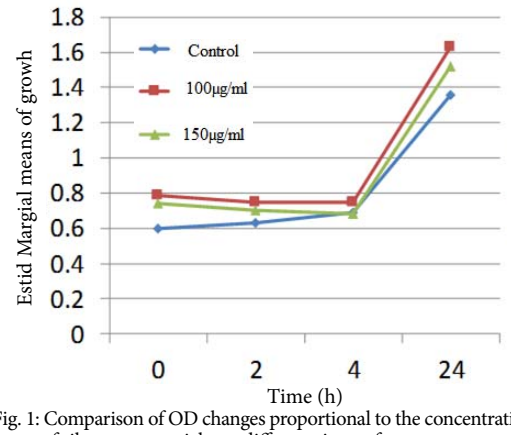

Fig. 1: Comparison of OD changes proportional to the concentration of silver nanoparticles at different times after treatment

Fig. 2: Image of electrophoresis for PCR products on agarose gel

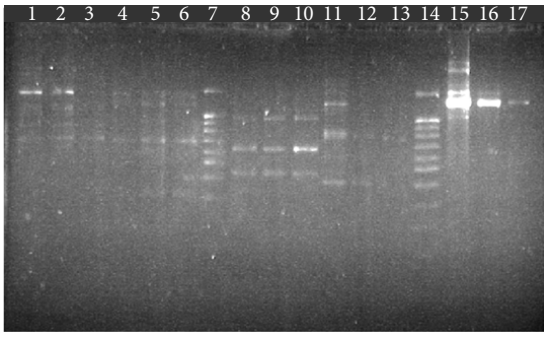

Fig. 3: Image of electrophoresis for PCR products on agarose gel

analysis were determined using markers. Samples were scored as one or zero based on the presence or absence of bands (Tables 1 and 2). The basis of conclusion was a difference among bands, for each primer forms for control samples and treated samples. As shown in Table 3, 11 respective primers produced 44 bands, among which 26 bands were different between the control and treated samples.

Based on data from RAPD, a similarity matrix was calculated for control and treated samples by DIC method that is shown in Table 4. The genetic distance between samples was ranged from 0.6333333 to 1.000 in which the numbers closer to one shows the more genetic similarity between samples.

Fig. 4 shows the dendrogram drawn by UPGMA method in NTSYS-PC software to compare the genetic variations between the control and the samples treated with silver nanoparticles. The control sample and treated samples were allocated separately in two main branches that explain the genetic differences.

Principal Coordinates Analysis (PCoA) was used to confirm the results obtained from cluster analysis. As seen in Fig. 5, control genotype is located in a group and treated genotypes in another group.

Table 2: Results of banding related to primers of Fig. 3

primers	band	C	T1	T2
OPA.9	1500	1	1	0
	1300	1	1	0
	800	1	1	0
OPR12	700	1	1	1
	1300	1	0	1
	700	1	1	1
OPT17	>1500	0	0	0
	1300	0	0	0
	900	0	1	1
	500	0	1	0
OPD.4	1000	1	1	1
	650	1	1	1
	450	1	1	1
OPR11	>1500	1	0	0
	1500	1	0	0
	1400	1	1	1
	1100	1	0	0

DISCUSSION

Infectious diseases caused by gram-positive strains of drug-resistant *S. aureus*, which usually have a nosocomial origin, are increasing in many countries. Therefore, the many efforts have been made to find new alternative compounds for antibiotics [2,3].

Generally, antibacterial agents are categorized into two bactericidal and bacteriostatic (growth inhibitor) groups. Antibacterial agents are used against infectious diseases. However, the common phenomenon of use and excessive use of antibacterial drugs has caused resistance to antibacterial drugs, which is an extraordinary and unexpected problem. Resistance is often

Table 3: Primer sequences

Nucleotide sequence of primer	Primer,s name	Number of formed bands	difference in control and treated bands
GTTTCGCTCC	OPB	1	0
ACCGCGAAGG	OPD	4	2
AGTCGGGTGG	OPS11	5	3
GAAGCCCTTG	OPQ17	5	5
TTTGGGGCCT	OPS05	3	2
CAATCGCCGT	OPA11	9	7
GGGTAACGCC	OPA09	4	3
ACAGGTGCGT	OPR12	2	1
TCTGGTGAGG	OPT17	3	0
TCTGGTGAGG	OPD04	4	2
GTAGCCGTCT	OPR11	4	1

Table 4: Similarity matrix based on DIC similarity coefficient

	C	T1	T2
C	1		
T1	0.6538462	1	
T2	0.6333333	0.7419355	1

causing changes in different essential stages of development. For example, antibiotics resistance during treatment is heritable. In addition, gene transfer through the conjugation, transduction, and transformation could be a possible cause of resistance [7] and leads to the bacterial development

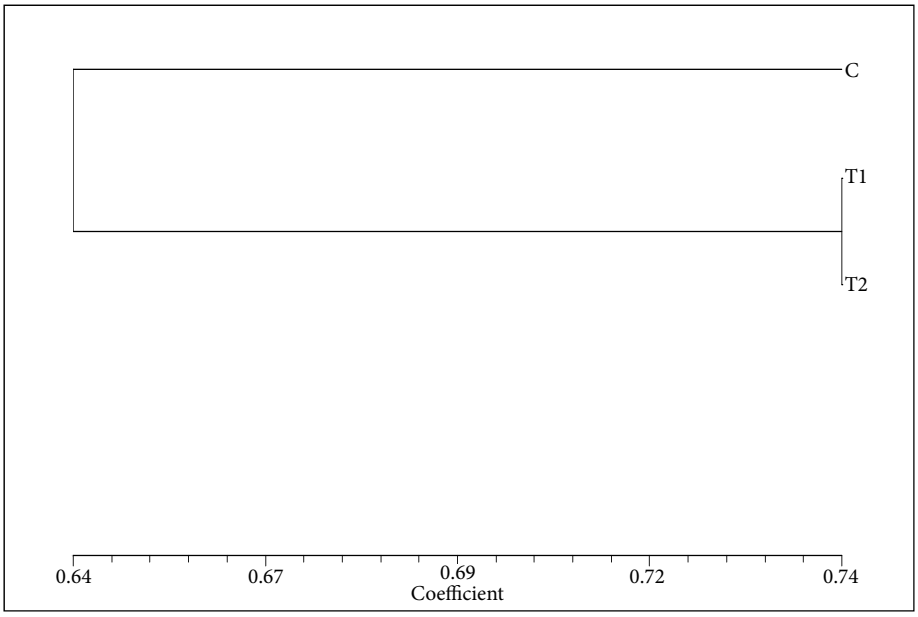

Fig. 4: Dendrogram obtained from analysis based on RAPD test via UPGMA method in NTSYS-PC software

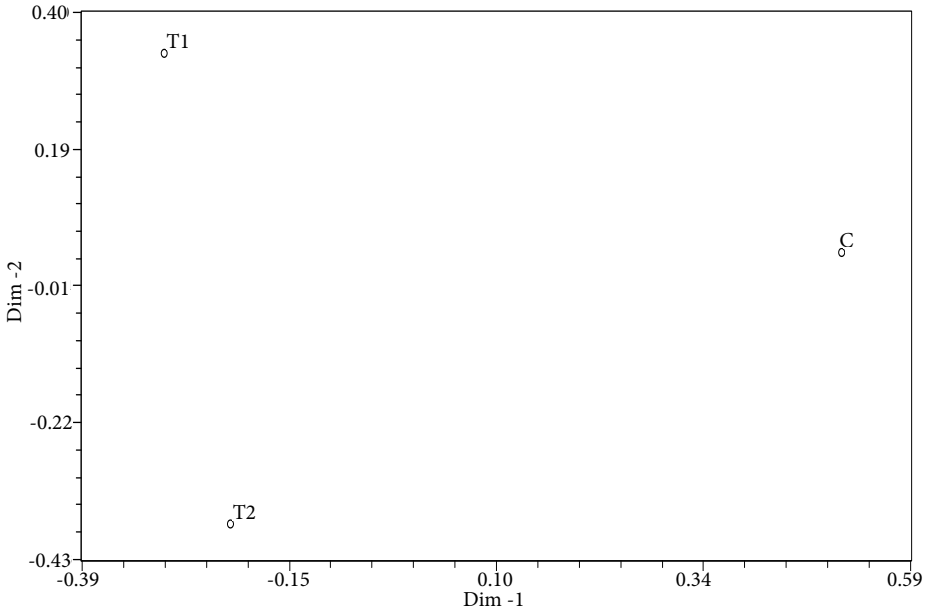

Fig. 5: PCoA graph

and continuation of the infectious disease. The intermittent development of alternative therapeutic strategies has raised the bacterial diseases[20]. The nanoparticles provide new antimicrobial agents [21]. According to the studies, the antibacterial activity of silver nanoparticles against a wide range of drug-resistant bacteria, viruses, and fungi such as *Candida albicans* has been proven in previous studies[4, 9, 14,17,21].

The results of a study on inhibitory properties of bacteria in different times and concentrations of treatment with silver nanoparticles on *S. aureus* in this study indicated that treatment of bacteria with different concentrations of silver nanoparticles after 4 hours caused significant changes on the reduction in studied bacteria at the concentrations of 100 and 150 µg/ml. Given that, many studies have proved antimicrobial effects of silver nanoparticles, and since heritability of antibiotic resistance in bacteria leads to multiple drug resistance (MDR) that are associated with plasmid gene, so the main objective of this study was to investigate the effects of silver nanoparticles on the genome of *S. aureus* as a model for gram-positive bacteria. Silver nanoparticles may affect the antibiotic resistance in bacteria by the antibacterial activity of the genes because nanoparticles could induce DNA single-strand break and affect the expression of the gene [22].

Li *et al.* [2012] reported that silver nanoparticle could penetrate to bacterial cells and affect DNA twisting, thus preventing replication and proliferation of the cell. Also, silver nanoparticles are combined with the thiol groups in the respiratory enzymes and could inhibit respiration process in bacterial cellular [23,24]. As shown in Table 3, 11 primers produced 44 bands in which 26 bands were different between the control and treated samples.

This difference indicates a change in the DNA sequence, which is caused by silver nanoparticles. As a result, a large number of primers did not detect target sequences. Therefore, the respective segment was not reproduced and its bands were not observed on an agarose gel. The difference between the bands observed in the treated and control groups of bacteria represents that the target sequences of primers have changed in the treated bacteria, which resulted in a difference in primers annealing and so segment amplification in PCR.

Changes in DNA sequence obtained in the present study could also be a factor to inhibit the growth and cell cycle through mutation and subsequently cause a change in gene expression associated with the growth and cell cycle control [25,26, 27]. Silver could release silver ions inside the cell that react with DNA phosphorus and disable DNA replication. Silver ions could increase ROS level, react with the sulfur-containing proteins, inhibit the respiratory enzymes and lead to the cell death [28,29,30]. Sondi *et al.* [2004] demonstrated that nanoparticles affect the enzymes involved in replication [31]; as a result, it can be concluded that bacteria in the growth and replication phase treated with silver nanoparticles in this study during replication and DNA repair mechanisms performing by the respective enzymes have been disrupted to cause multiple mutations in the DNA sequence .The results observed in this study also demonstrate reduced bacterial growth and confirm the mentioned statement. This conclusion needs further research.

CONCLUSION

The results of the present study indicated the efficacy of silver nanoparticles at the lowest dose and shortest time as an antibacterial agent. In addition, genetic diversity between control and treated bacteria demonstrated the silver nanoparticles effect on the genome of *S. aureus* as a model for gram-positive bacteria.

However, it is necessary to conduct further studies on the use of nanoparticles. Accordingly, nanoparticles affect bacterial DNA and cause mutations. The release of ions and subsequently ROS production are the main reasons of cytotoxic effect on most organisms including prokaryotes and eukaryotes cells. The nanoparticles could probably affect the human body and other organisms when using in large amounts or repeatedly in various industries, such as food and medicine, make mutations and eventually cancer. It is suggested to be conducted future studies investigating the effects of using nanoparticles on eukaryotic cells and genome as well as its effects on cancerous cells, so nanoparticles could be used more confidently in a variety of industries including food and medical industries.

ACKNOWLEDGEMENTS

Hereby, we would like to sincerely thank the Department of Biotechnology, Faculty of Science, University of Maragheh, Iran, for providing the nanoparticles and primers used in this study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Ravipaty S, Reilly JP. Comprehensive characterization of methicillin-resistant Staphylococcus aureus subsp. aureus COL secretome by two-dimensional liquid chromatography and mass spectrometry. Molecular & Cellular Proteomics. 2010;9(9):1898-919.
2. Chambers HF. The changing epidemiology of Staphylococcus aureus? EMERG INFECT DIS. 2001;7(2):178-82.
3. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure. Antimicrob Agents Chemother. 2005;49(3):1222-4.
4. Blecher K, Nasir A, Friedman A. The growing role of nanotechnology in combating infectious disease. Virulence. 2011;2(5):395-401.
5. Fahmy B, Cormier SA. Copper oxide nanoparticles induce oxidative stress and cytotoxicity in airway epithelial cells. Toxicol in Vitro. 2009;23(7):1365-71.
6. Amelia M, Lincheneau C, Silvi S, Credi A. Electrochemical properties of CdSe and CdTe quantum dots. Chem Soc Rev. 2012;41(17):5728-43.
7. Witte W. International dissemination of antibiotic resistant strains of bacterial pathogens. Infection, Genetics and Evolution. 2004;4(3):187-91.
8. Rabolli V, Thomassen LCJ, Uwambayinema F, Martens JA, Lison D. The cytotoxic activity of amorphous silica nanoparticles is mainly influenced by surface area and not by aggregation. Toxicol Lett. 2011;206(2):197-203.
9. Landini P, Antoniani D, Burgess JG, Nijland R. Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl Microbiol Biotechnol. 2010;86(3):813-23.
10. Lara HH, Ayala-Núñez NV, Ixtepan Turrent LdC, Rodríguez Padilla C. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J Microbiol Biotechnol. 2010;26(4):615-21.
11. Sutherland IW. Biofilm exopolysaccharides: a strong and sticky framework. MICROBIOLOGY+. 2001;147(1):3-9.
12. Juan L, Zhimin Z, Anchun M, Lei L, Jingchao Z. Deposition of silver nanoparticles on titanium surface for antibacterial effect. Int J Nanomed. 2010;5:261-7.
13. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv. 2009;27(1):76-83.
14. Knetsch MLW, Koole LH. New Strategies in the Development of Antimicrobial Coatings: The Example of Increasing Usage of Silver and Silver Nanoparticles. Polymers. 2011;3(1):340-66.
15. Martinez LR, Han G, Chacko M, Mihu MR, Jacobson M, Gialanella P, et al. Antimicrobial and Healing Efficacy of Sustained Release Nitric Oxide Nanoparticles Against Staphylococcus Aureus Skin Infection. J, Invest Dermatol. 2009;129(10):2463-9.
16. Périchon B, Courvalin P. VanA-type vancomycin-resistant Staphylococcus aureus. Antimicrob Agents Chemother. 2009;53(11):4580-7.
17. Hindi KM, Ditto AJ, Panzner MJ, Medvetz DA, Han DS, Hovis CE, et al. The antimicrobial efficacy of sustained release silver–carbene complex-loaded l-tyrosine polyphosphate nanoparticles: Characterization, in vitro and in vivo studies. BIOMATERIALS. 2009;30(22):3771-9.
18. Huang L, Dai T, Xuan Y, Tegos GP, Hamblin MR. Synergistic combination of chitosan acetate with nanoparticle silver as a topical antimicrobial: efficacy against bacterial burn infections. Antimicrob Agents Chemother. 2011;55(7):3432-8.
19. Brown AN, Smith K, Samuels TA, Lu J, Obare SO, Scott ME. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. Appl Environ Microbiol. 2012;78(8):2768-74.
20. Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. TRENDS MICROBIOL. 2006;14(4):176-82.
21. Huh AJ, Kwon YJ. "Nanoantibiotics": A new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Controlled Release. 2011;156(2):128-45.
22. Chang Y-N, Zhang M, Xia L, Zhang J, Xing G. The Toxic Effects and Mechanisms of CuO and ZnO Nanoparticles. Materials. 2012;5(12).
23. Klasen HJ. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. BURNS. 2000;26(2):131-8.
24. Li W-R, Xie X-B, Shi Q-S, Duan S-S, Ouyang Y-S, Chen Y-B. Antibacterial effect of silver nanoparticles on Staphylococcus aureus. BIOMETALS. 2011;24(1):135-41.
25. Durán N, Durán M, de Jesus MB, Seabra AB, Fávaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomed Nanotechnol Biol Med. 2016;12(3):789-99.
26. Chowdhury NR, MacGregor-Ramiasa M, Zilm P, Majewski P, Vasilev K. 'Chocolate' silver nanoparticles: Synthesis, antibacterial activity and cytotoxicity. J Colloid Interface Sci. 2016;482(Supplement C):151-8.
27. Iniyan AM, Kannan RR, Joseph F-JRS, Mary TRJ, Rajasekar M, Sumy PC, et al. In vivo safety evaluation of antibacterial silver chloride nanoparticles from Streptomyces exfoliatus ICN25 in zebrafish embryos. MICROB PATHOGENESIS. 2017;112(Supplement C):76-82.
28. Feng QL, Wu J, Chen G, Cui F, Kim T, Kim J. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J BIOMED MATER RES. 2000;52(4):662-8.
29. Matsumura Y, Yoshikata K, Kunisaki S-i, Tsuchido T. Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. Appl Environ Microbiol. 2003;69(7):4278-81.
30. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. NANOTECHNOLOGY. 2005;16(10):2346.
31. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci. 2004;275(1):177-82.