Journal of
Nano Drug Science

http://jnds.zbmu.ac.ir Original Article

2018, Volume 1, Issue 1, p. 10-13

# Evaluating the Antimicrobial Activity of Zinc Oxide Nanoparticles Against Gram-Positive and Gram-Negative Bacteria in Aquatic Animals

**Hadi Mirzaei[1], Mahin Rigi[2*], Hamid Reza Jahantigh[3]**

[1]Zabol Medicinal Plant Research Center, Zabol University of Medical Sciences, Zabol, Iran
[2]Department of Fisheries, Hamoun International Wetland Research Institute, University of Zabol, Zabol, Iran
[3]Department of Agriculture, University of Zabol, Zabol, Iran

***Corresponding author:** Mahin Rigi, Email: mahinrigi255@gmail.com

**Abstract**

**Introduction:** Considering the resistance of bacteria to different types of antibiotics and conventional antimicrobial agents, many studies have been conducted to find new types of effective antimicrobial agents. The purpose of this study was to evaluate the antimicrobial activity of zinc oxide nanoparticles against gram-positive and gram-negative bacteria in aquatic animals.

**Methods:** Zinc oxide nanoparticles were commercially-purchased. The bacteria studied were purchased in ampoules from the company. Finally, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration were determined by the microdilution method.

**Results:** According to the results of this study, the maximum inhibitory concentration of zinc oxide nanoparticles was observed against *Staphylococcus aureus* (1500 μg/mL). The MIC was found to be 187 μg/mL against Shigella dysenteriae and the maximum bactericidal concentration (MBC) was observed against *S. aureus*, *Bacillus cereus* and *Listeria monocytogenes* (1500 μg/mL). However, the minimum bactericidal concentration was observed against *Shigella dysenteriae*.

**Conclusion:** The results of this study showed that zinc oxide nanoparticles inhibited the studied bacteria, which increased with increasing inhibitory concentration.

**Keywords:** Zinc nano oxide, Antimicrobial activity, Aquaculture

Received: 7 August 2018, Accepted: 20 November 2018, ePublished: 17 December 2018

## Introduction

The use of appropriate antibacterial agents can always control potentially pathogenic factors, which reduces the contamination and disease of crops and thus reduces the need for antibiotics. In the aquaculture industry, in addition to the problem of drug resistance, antibiotic residues are also transmitted to human through consumption, therefore, the introduction of disinfectants instead of antibiotics, especially in aquatic products seems very necessary.[1]

Disinfectants are usually used to control infections caused by pathogens in fish farms. These compounds should be used at concentrations that are not fatal to fish. The effect of an antiseptic agent depends on the amount of time and concentration of that substance. In this case, the fish should be placed in water with an appropriate amount of time and concentration to eliminate infectious organisms without being damaged.[2]

Today, nanotechnology has a significant impact on various fields of industry, health, medicine, and food. The beneficial properties of nanomaterials have led to their use in various and sensitive processes, particularly in biology and pharmaceutical applications, and have attracted the attention of biologists and pharmacists to the application of nanomaterials in food and pharmaceutical industries. There are reports of antibacterial and antiviral effects of nanomaterials. It is reported that one of the ways to fight the AIDS virus is to use nanomaterials. Because nanomaterials, including heavy metal oxide, tend to be highly reactive with biological molecules and cause them to be inactivated and eventually destroy the virus or bacteria.[3,4]

Nanomaterials exhibit the lowest toxicity levels in the life cycle and ecosystems, so using these materials to counteract pathogenic microbes can be a good choice. Studies have shown that nanoparticles such as Zn, Ti, Ag, Cr and their oxides have a high bactericidal effect.[4]

One of the most important nanoparticles is zinc oxide, which is used on industrial scale in many countries.[5] The mechanism of zinc oxide is similar to other nanoparticles but it is more effective in destroying the bacterial wall. The main mechanism of the effect of nanoparticles on bacteria is through damaging DNA and proteins and destructing cell wall.[6]

Zinc is a metal that is widely distributed in nature and is essential for the function of many metalloproteins. Zinc oxide nanoparticles have antimicrobial effects and have advantages over silver nanoparticles, including lower prices, white appearance, and the ability to block violet radiation.[7]

The antimicrobial effects of zinc oxide nanoparticles have been proven on a wide range of gram-positive and gram-negative bacteria, including *Staphylococcus aureus, Enterococcus, Salmonella typhimurium,* and *Enterobacter aerogenes.*

The purpose of this study was to evaluate the antimicrobial activity of zinc oxide nanoparticles against gram-positive and gram-negative bacteria in aquatic animals.

## Methods

### Preparation of Zinc Oxide Nanoparticles

Zinc oxide nanoparticles are commercially purchased from Iranian Nanomaterials Pioneers Company. The size of the nanoparticles is 10-30 nm with a molecular weight of 81/37.

### Preparation and Storage of Bacteria

The strains of *Vibrio cholera, Micrococcus luteus, Staphylococcus epidermis, Bacillus cereus* and *Listeria monocytogenes* were prepared from Iranian fungal and bacterial collection. They were incubated for 24 hours at 37°C in a nutrient broth medium. After 24 hours, they were stored in a broth culture medium containing 10% sterilized glycerol in a freezer at -20°C for later use.

### Preparation of Bacterial Suspension

*Vibrio cholera, M. luteus, S. epidermis, B. cereus,* and *L. monocytogenes* were cultured in a nutrient broth medium after thawing at 37°C in order to differentiate pure colonies from bacterial samples on a solid TSA medium and were incubated for 24 hours at 37°C. After 24 hours, the pure colonies of each bacterium were removed and 0.5 McFarland opacity was achieved. Then, its absorption with a wavelength of 600 nm was read using the UV visible spectrophotometer to ensure the concentration of bacteria. The density of bacteria with $1/5*10^6$ CFU/mL has an absorption of 0.08-0.1.

### Determining the Sensitivity of Bacterial Strains to Zinc Oxide Nanoparticles

The susceptibility of bacterial isolates to zinc oxide nanoparticles was determined in the wells by the dilution method. Six wells were created in a solid culture medium and 100 µL of each well was added to the Mueller-Hinton broth (MHB). Then, 100 mL of a diluted solution of the extracts of plants was added to the first well, and after mixing, 100 mL of the suspension in the first well was removed and added to the second well, and this was done up to the last well. 100 µl of the suspension in the culture medium was removed from the last well and 10 µL of a microbial suspension containing 107 units/mL, equivalent to 0.5 McFarland, was added to it and incubated at 37°C for 24 hours. The first wells in which the growth of the bacteria was prevented after being inserted into the incubator were considered as the minimum inhibitory concentration (MIC). Ten microliters of the suspension in the transparent wells was removed and transferred to the Mueller-Hinton agar medium, and after 24 hours, the first dilution that could destroy 99.9% of the bacteria was shown as the minimum bactericidal concentration.

## Results

According to the results of this study, the maximum inhibitory concentration of zinc oxide nanoparticles was observed against *S. aureus* (1500 µg/mL). The MIC was found to be 187 µg/mL against *Shigella dysenteriae*. The maximum bactericidal concentration (MBC) was observed against *S. aureus, B. cereus* and *L. monocytogenes* (1500 µg/mL). However, the minimum bactericidal concentration was observed against *S. dysenteriae* (Table 1).

## Discussion

In a study by Hoseinzadeh et al, the antimicrobial activity of zinc oxide nanoparticles against gram-positive and gram-negative bacteria was investigated. The results of the study of nanoparticle properties using X-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) showed that particle size was within the range and there was no impurity in nanoparticle crystals. The diameter of the zinc oxide nanoparticles was 20 nm. The diameter of the growth halo of *Pseudomonas aeruginosa* in Zao was larger compared to the other strains. The population of *P. aeruginosa* has reached zero at a concentration of 2X MIC for 150 minutes in the presence of ZnO.[8]

According to Atmaca et al, zinc oxide and zinc acetate have an antimicrobial effect on *S. aureus*, *Staphylococcus epidermidis* and *P. aeruginosa* in the MHB. The effect of zinc oxide on *S. aureus* and *S. epidermidis* was higher compared to *P. aeruginosa*.[9]

Xie et al concluded that the MIC for *Campylobacter*

**Table 1.** Results of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Zinc Oxide Nanoparticles on Gram-Positive and Gram-Negative Bacteria (µg/mL)

| Bacteria | MIC | MBC |
|---|---|---|
| *Staphylococcus aureus* | 1500 | 1500 |
| *Bacillus cereus* | 750 | 1500 |
| *Vibrio cholera* | 375 | 750 |
| *Listeria monocytogenes* | 750 | 1500 |
| *Shigella dysenteriae* | 187 | 375 |

*jejuni* varied from 0.05 to 0.025 mg/mL and zinc oxide nanoparticles at this concentration for *C. jejuni* are not only bacteriostatic but also have bactericidal activity.[10]

The results of Sinha et al showed that zinc oxide nanoparticles decreased the growth rate of Enterobacter strains by 50% and halophilic bacteria by 80%.[11]

In the study of Ismail Zadeh et al, who investigated the effect of zinc oxide nanoparticles on the growth of *Bacillus subtilis* and *E. coli*, the results showed that zinc oxide nanoparticles at both concentrations of 2% and 4% significantly reduced the growth of *E. coli*. However, in the case of *B. subtilis* bacteria, only concentration of 4% significantly reduced the number of bacteria and no significant change was observed in the number of *B. subtilis* bacteria at a concentration of 2%.[12]

Emamifar et al also investigated the effects of polyethylene packaging containing 1.5% and 5% silver nanoparticles and 25% and 1% zinc oxide nanoparticles in the inactivation of *Lactobacillus plantarum* in orange juice. They found that all nanocomposites except the nanocomposite containing 1% zinc oxide could reduce the growth of bacteria.[13]

Zhang et al examined various factors such as the size and concentration of zinc oxide nanoparticles on the intensity of antimicrobial activity against *E. coli*. They concluded that nanoparticle concentrations played an important role in their size and the antimicrobial effect was increased by increasing the concentration of nanoparticles.[14]

Li et al investigated the antimicrobial activity of zinc oxide nanoparticles, which were coated with polyvinyl chloride film, on gram-positive bacteria of *S. aureus* and *E. coli*. They reported that zinc oxide nanoparticles are more effective against gram-positive bacteria compared to gram-negative bacteria.[15]

In a study by Veissi Malekshahi et al, who investigated the antimicrobial activity of zinc oxide nanoparticles, the results showed that by increasing the number of plasma rounds, the antioxidant activity of zinc oxide increased and the number of colonies decreased.[16]

Hoseinzadeh et al studied the synergistic effect of commercial nanoparticles of copper oxide and zinc oxide against gram-positive and gram-negative bacteria using fractional inhibitory concentration index. The results showed that the MIC of *E. coli* and *S. epidermidis* was lower in the combined state of nanoparticles compared to the single state. The fractional inhibitory concentration index for *E. coli*, *S. aureus*, *P. aeruginosa* and *S. epidermis* was 0.75, 9.6 and 0.625, respectively.[17]

Yang et al stated that the photocatalytic ability of zinc oxide nanoparticles in combination with silver nanoparticles increases and improves the oxidation of silver nanoparticles. In addition, the ability to regenerate silver nanoparticles is enhanced due to the photocatalytic properties of Zinc oxide nanoparticles. This inhibits the growth of bacteria.[18]

In a study by Nasiri et al, the antimicrobial properties of zinc oxide nanoparticles synthesized by ultrasonic waves were analyzed. The results showed that the MIC of bacterial growth for *E. coli* was 0.125 mg/mL and for *S. aureus* it was 0.062 mg/mL. The minimum bacterial concentration for *E. coli* was 0.500 mg/mL and for *S. aureus* it was 0.250 mg/mL. The inhibition zone diameter for *E. coli* was 1.527 ± 18.66 mm and for *S. aureus* it was 24.66 ± 0.577 mm.[19]

Jiang et al reported that zinc oxide showed stronger antibacterial activity in comparison with the metal oxides such as AI2O3, TiO2 and SiO2 against *E. coli, Pseudomonas fluorescens* and *B. subtilis*.[20]

Reddy et al investigated the antimicrobial effects of zinc oxide nanoparticles on *S. aureus* and *E. coli* in India. They observed that the gram-positive bacteria of *S. aureus* are more sensitive to zinc oxide nanoparticles than gram-negative bacteria of *E. coli*.[21]

In another study that investigated the effect of zinc oxide nanoparticles on the kinetics of the death of gram-positive and gram-negative bacteria, the results showed that increasing the concentration of nanoparticles in all strains reduced the survival ratio. By plotting the logarithm of bacteria survival versus contact time, the number of all bacterial species decreased linearly with time and the death rate was constant with increasing contact time and the concentration of nanoparticle suspension increased from 1 to 2 MIC.[22]

In a study by Mohammadbeigi et al who examined the antimicrobial activity of zinc oxide nanoparticles on *Streptococcus iniae* and *E. coli*, the results showed that the MBC values of zinc oxide nanoparticles for *S. iniae* and *E. coli* were 0.095 and 0.6 μg/mL, respectively. Moreover, the MIC values of this substance for *S. iniae* and *E. coli* were 0.015 and 0.95 μg/mL, respectively.[23]

## Conclusion

The results of this study showed that zinc oxide nanoparticles have an appropriate antibacterial effect on *V. cholera, M. luteus, S. epidermidis, B. cereus* and *L. monocytogenes*. Therefore, it can be used as an appropriate option for disinfecting the aquarium environment.

## Conflict of Interests

None.

## References

1. Hahm DH, Yeom MJ, Lee EH, Shim I, Lee HJ, Kim HY. Effect of *Scutellariae radix* as a novel antibacterial herb on the ppk (polyphosphate kinase) mutant of *Salmonella typhimurium*. J Microbiol Biotechnol. 2001;11(6):1061-1065.
2. Moshtaqi B, Nezami SH, Khara H, et al. Determining of fatal density of KmNo4 and Cuso4H2o in Acipenser persicus Bordin. Biology Magazine. 2009;3(4):67-78.
3. Burda C, Chen X, Narayanan R, El-Sayed MA. Chemistry and properties of nanocrystals of different shapes. Chem Rev. 2005;105(4):1025-1102. doi:10.1021/cr030063a
4. Li P, Li J, Wu C, Wu Q, Li J. Synergistic antibacterial effects

of lactam antibiotic combined with silver nanoparticles. Nanotechnol. 2005;16(9):1912-1917. doi:10.1088/0957-4484/16/9/082

5. Heinlaan M, Ivask A, Blinova I, Dubourguier HC, Kahru A. Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. Chemosphere. 2008;71(7):1308-1316. doi:10.1016/j.chemosphere.2007.11.047
6. Llorens A, Lloret E, Picouet PA, Trbojevich R, Fernandez A. Metallic-based micro and nanocomposites in food contact materials and active food packaging. Trends Food Sci Technol. 2012;24(1):19-29. doi:10.1016/j.tifs.2011.10.001
7. Hoseinzadeh E, Alikhani MY, Samarghandy MR. Evaluation of synergistic effect of commercial zinc oxide and copper oxide nanoparticles against gram positive and gram negative bacteria by fraction inhibitory concentration index. Journal of Zanjan University of Medical Sciences and Health Services. 2012;20(82):31-43. [Persian].
8. Atmaca S, Gul K, Clcek R. The effect of zinc on microbial growth. Turk J Med Sci. 1998;28(6):595-597.
9. Xie Y, He Y, Irwin PL, Jin T, Shi X. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. Appl Environ Microbiol. 2011;77(7):2325-2331. doi:10.1128/aem.02149-10
10. Sinha R, Karan R, Sinha A, Khare SK. Interaction and nanotoxic effect of ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. Bioresour Technol. 2011;102(2):1516-1520. doi:10.1016/j.biortech.2010.07.117
11. Esmailzadeh H, Sangpour P, Khaksar R, Shahraz F. The effect of ZnO nanoparticles on the growth of *Bacillus subtilis* and *Escherichia coli* O157: H7. J Food Technol Nutr. 2014;11(3):21-28.
12. Emamifar A, Kadivar M, Shahedi M, Soleimanian-Zad S. Effect of nanocomposite packaging containing Ag and ZnO on inactivation of *Lactobacillus plantarum* in orange juice. Food Control. 2011;22(3):408-413. doi:10.1016/j.foodcont.2010.09.011
13. Zhang L, Jiang Y, Ding Y, Povey M, York D. Investigation into the antibacterial behaviour of suspensions of ZnO nanoparticles (ZnO nanofluids). J Nanopart Res. 2007;9(3):479-489. doi:10.1007/s11051-006-9150-1
14. Li H, Li F, Wang L, et al. Effect of nano-packing on preservation quality of Chinese jujube (*Ziziphus jujuba* Mill. var. inermis (Bunge) Rehd). Food Chem. 2009;114(2):547-552. doi:10.1016/j.foodchem.2008.09.085
15. Veissi Malekshahi Z, Afshar D, Ranjbar R, et al. Antimicrobial properties of ZnO nanoparticles. Quarterly Journal of Infectious Diseases and Tropical Medicine. 2012;17(59):1-4.
16. Hoseinzadeh E, Samarghandi MR, Alikhani M, Asgari G, Roshanaei G. Effect of zinc oxide (ZnO) nanoparticles on death kinetic of gram-negative and positive bacterium. J Babol Univ Med Sci. 2012;14(5):13-19. [Persian].
17. Yang L, Mao J, Zhang X, et al. Preparation and characteristics of Ag/nano-ZnO composite antimicrobial agent. Nanoscience. 2006;11(1):44-48.
18. Nasiri A, Malakootian M, Tamaddon F. Synthesis nano ZnO assisted by ultrasound irradiation and evaluation of antimicrobial properties. Toloo e behdasht. 2014;13(4):115-128. [Persian].
19. Jiang W, Mashayekhi H, Xing B. Bacterial toxicity comparison between nano- and micro-scaled oxide particles. Environ Pollut. 2009;157(5):1619-1625. doi:10.1016/j.envpol.2008.12.025
20. Reddy KM, Feris K, Bell J, Wingett DG, Hanley C, Punnoose A. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett. 2007;90(213902):2139021-2139023. doi:10.1063/1.2742324
21. Hoseinzadeh E, Samargandi MR, Alikhani MY, Roshanaei G, Asgari G. Antimicrobial efficacy of zinc oxide nanoparticles suspension against gram negative and gram positive bacteria. Iran J Health Environ. 2012;5(3):331-342.
22. Mohammadbeigi P, Sodagar M, Mazandarani M, Hoseini SS. An investigation of antibacterial activity of ZnO nanoparticle on *Streptoccocus iniae* and *Escheria coli*. Qom Univ Med Sci J. 2016;10(5):55-63.
23. Handy RD, Cornelis G, Fernandes T, et al. Ecotoxicity test methods for engineered nanomaterials: practical experiences and recommendations from the bench. Environ Toxicol Chem. 2012;31(1):15–31. doi: 10.1002/etc.706