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Evaluation of the Effect of Zinc Nano Oxide on *Salmonella typhimurium* Poultry Isolates

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Abstract

Introduction: The aim of this study was to evaluate the effect of nano oxide on *Salmonella typhimurium* isolated from poultry in Zabol.

Methods: Zinc nano oxide was purchased. The *S. typhimurium* was isolated from poultry in Zabol and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the microdilution method.

Results: The results of this study showed that the MIC against S. typhimurium was 93 µg/mL, and 3 strains were inhibited in this concentration. In addition, the maximum inhibitory concentration was 3000 µg/mL, and one strain was inhibited in this concentration as well.

Conclusion: The results of this study demonstrated good antimicrobial effects of zinc oxide nanoparticles on *S. typhimurium,* which can be used to treat infections caused by *S. typhimurium*.

Keywords: Antimicrobial activity, Zinc nano oxide, *Salmonella typhimurium*, Poultry

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Introduction

In recent years, bacteria have become very resistant to antibiotic treatments. The speed of making a stronger antibiotic to be replaced is by no means responsive to the speed of increased bacterial resistance. Hence, the urgent need for new approaches to confront bacterial infections is felt. One way to deal with these microbial agents is to replace antibacterial factors so that different microbial species do not become resistant to these factors. These antimicrobial agents include metal nanoparticles that have been commonly studied in recent years.[1]

Zinc nano oxide nanoparticles are used in the electronics, textiles, cosmetics, sprays, chemical sensors, and food packaging.[2,3] These nanoparticles have a strong antimicrobial activity against gram-positive and gram-negative bacteria, especially foodborne illness bacteria such as *Escherichia coli, Listeria monocytogenes, Salmonella*, and *Staphylococcus aureus*.[4] Studies have shown that nanoparticles have higher cytotoxicity than metal ions since they can penetrate into the cell membrane and release metal ions within the cell.[5] One of the most common diseases is salmonellosis, which is caused by different serotypes of *Salmonella* bacteria, and there are concerns about the contamination of poultry and its products by this microorganism.[6] *Salmonella* is one of the major sources of foodborne diseases in many parts of the world causing mild gastroenteritis to lethal septicemia in humans. Infections in humans are caused by the consumption of contaminated foods such as vegetables, dairy products as well as marine and meat products especially poultry meat, eggs, and by-products.[7] Meat and poultry eggs and their products have always been the main sources of *Salmonella* in foodborne illnesses.[8]

The aim of this study was to evaluate the effect of zinc nano oxide on *Salmonella typhimurium* isolated from poultry in Zabol.

Methods

Preparation of Zinc Oxide Nanoparticles

Nanoparticles of zinc oxide with a size of 10-30 nm were commercially purchased from the Iranian nanomaterials pioneer's company (Iranian Nanomaterials Ploneers Company).

Isolation of Bacteria

The samples of poultry feces were collected from Zabol in 2013. Samples were stored in the environment for 6 hours. They were then transferred to selective media of *Salmonella, Shigella* and Simon sulfite agars and kept at 37°C for 24 hours. The obtained samples were tested for the final detection of *S. typhimurium* species by biochemical tests and differential cultures such as TSI,

MRVP, lysine iron agar, Simmons' citrate agar, and Urea *Salmonella typhimurium* because *Salmonella* specimens grow only on these environments.

Preparation of 0.5 McFarland Suspension

In order to prepare a microbial suspension, 24 hours before the test, a bacterial storage medium was first inoculated into a sloped agar culture medium. After the growth of bacterial colonies, the culture medium was washed with a normal saline solution and a concentrated microbial suspension was obtained. Then, some bacterial suspensions were inserted into a sterile tube containing a normal saline solution and its opacity was measured with a spectrophotometer (Unico-American) at a wavelength of 630 nm and diluted with the normal saline solution until the opacity of the MacFarland 0.5% opacity was equilibrated. The bacterial suspension was prepared with a concentration of 1×10^8 CFU/mL.

Determining the Sensitivity of Bacteria to Conventional Antibiotics

The susceptibility of bacterial strains to AM, GM, CZ, AMC, and AZM antibiotics (Padtan Teb, Iran) were evaluated using standard Kirby-Bauer diffusion method. For this purpose, first, all bacterial strains were prepared at a concentration of 0.5 McFarland in the Mueller-Hinton medium and then were spread and cultured on Mueller-Hinton agar (MHA) medium. Antibiotic disks were placed at an appropriate distance and the plates were kept in an incubator for 24 hours at 37°C.The diameter of the inhibitory holes was also measured to determine the resistance and susceptibility of the strains to the desired antibiotics.

Determining the Sensitivity of 12 Strains of *Salmonella typhimurium* to Zinc Nano Oxide

Determining the susceptibility of bacterial isolates to zinc nano oxide was carried out by dilution method in wells. Six wells were created in a solid culture medium and 100 µL of each well was added to the nutrient medium of Muller-Hinton Broth (MHB). Then, 100 mL of a diluted solution of the plant extracts were added to the first well and after mixing, 100 µL of the first well was added to the second well, and this trend was continued and similarly carried out for the last well, as well. Then, 100 µL of the culture medium was removed from the last well and 10 µL of the microbial suspension containing 107 units per ml, equivalent to 0.5 McFarland, was added to the incubator at 37°C for 24 hours. The first wells that prevented the growth of the bacteria after insertion into the incubator, were considered as the minimum inhibitory concentration (MIC). To ensure that the wells were clear, 10 µL was removed and transferred to the MHA medium. And after 24 hours, the first dilution that could destroy 99.9% of the bacteria was shown as the minimum bactericidal concentration (MBC).

Results

The results of this study indicated that the MIC against *S. typhimurium* was 93 µg/mL, and 3 strains were inhibited in this concentration. The maximum inhibitory concentration was 3000 µg/mL, one strain of which was inhibited in this concentration.

The lowest bactericidal concentration was 187 µg/mL and 4 strains were lost in this concentration. The maximum bactericidal concentration was 3000 µg/mL (Table 1).

The results of antibiotic resistance evaluation showed that *S. typhimurium* was susceptible to gentamicin antibiotics, while multiple strains were resistant to ampicillin antibiotics (Table 2).

Discussion

Rezaie Keikhaie carried out a study in order to evaluate the antimicrobial effects of zinc oxide nanoparticle and extract of *Solanum nigrum* on *Pseudomonas aeruginosa* bacteria isolated from clinical specimens. The highest inhibitory concentration for *P. aeruginosa* was 1500 µg/mL, with 4 bacterial strains being inoculated. In addition, the results demonstrated that the highest drainage concentration was 3000 µg/mL, and that 2 strains were inhibited in this concentration and the lowest trap concentration was 93 µg/mL as well. The lowest inhibitory concentration of extract plant was 0.62 mg/mL, with only one strain being inhibited in this concentration. The highest inhibitory concentration for *P. aeruginosa* was 40 mg/mL, with 4 bacterial strains being inoculated.[9]

Golestani et al in their study investigating the effects of copper oxide nanoparticles on the genome of *S. typhimurium* using the RAPD (random amplified polymorphic DNA) molecular marker, found that copper oxide nanoparticles cause sequencing changes in different parts of the genome of *Salmonella* bacteria at concentrations of 90 and 120 µg/mL.[10]

Naddafi et al also investigated the toxicity of nanoparticles of zinc oxide and titanium oxide using biological tests by *E. coli* and *S. aureus*. The results showed that 24-hour EC50 Nano ZnO was found to be 5.47 mg/mL

Table 1. MIC and MBC Nano Silver Against *Salmonella typhimurium*

Bacterial Code	MIC	MBC
1	375	375
2	1500	3000
3	93	187
4	375	750
5	750	1500
6	375	375
7	750	1500
8	93	187
9	3000	3000
10	375	750
11	750	750
12	93	187

Table 2. Antibiotic Resistance Pattern of *Salmonella typhimurium* Strains

Bacterial Strain	AM	GM	AZM	AMC	CZ
1	R	S	I	I	I
2	S	S	S	S	S
3	R	S	S	S	I
4	S	S	S	S	S
5	S	S	S	S	S
6	R	S	S	I	S
7	R	S	S	S	I
8	I	S	S	S	S
9	I	S	S	S	I
10	R	S	S	S	S
11	I	S	S	S	S

and 2.38 mg/mL using *E. coli* and *S. aureus*, respectively. Furthermore, the concentration without inhibitory effect was found to be 1.15 and 3.28 mg/mL using *E. coli* and *S. aureus*, respectively.[11]

In the study conducted by Barzegary Firouzabadi et al, suspension treatment (0-1-3-5-8 mM) of zinc oxide nanoparticles in malic acid was found to have a significant inhibitory effect on the growth of *E. coli* and *S. aureus* during 24 hours incubation. The results indicated that the concentration of 5 and 8 mM of zinc oxide nanoparticles with malic acid had the highest inhibitory effect in both strains. The suspension of zinc oxide nanoparticles and malic acid had the preferential ability to suppress the growth of *E. coli* and *S. aureus* in carrot juice.[12]

Abbaspour et al examined the effect of sterile gas composition with chitosan nanocomposite/silver/zinc oxide on microbial properties. The results showed that the sterile gas sample obtained from chitosan and zinc oxide reduced the number of colonies from 1000 to 30 in the raw sample.[13]

Similarly, in another study by Badiei et al, in which the synthesis of zinc oxide nanoparticles was investigated using ionic liquid and their antimicrobial properties, the results indicated that zinc oxide was produced from spherical nanoparticles with a mean size of 45 nm. The sample was also porous and the specific zinc oxide surface was almost pure and only very little impurity of zinc acetate was observed in the infrared spectrum of the sample. The antimicrobial properties of the sample on *E. coli* were investigated and the results showed that zinc oxide nanoparticles had high antibacterial activity.[14]

Zinc oxide nanoparticles showed a broad range of antimicrobial activity, inhibiting the growth of *E. coli* and *L. monocytogenes* in concentrations above 0.24 mg/mL.[15,16]

Zinc oxide nanoparticles had an inhibitory effect on *Bacillus subtilis, S. aureus, S. epidermidis, S. pyogenes*, and *E. faecalis* bacteria.[17]

In a study by Dobrucka and Diugazewska's on investigation of the antimicrobial activity of zinc oxide nanoparticles synthesized in *Trifolium pratense* flower extract, the results showed that these nanoparticles had an antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli*.[18]

In the same vein, in their study, Emami-Karvani and Chehrazi investigated the antimicrobial effect of zinc nano oxide on gram-positive and gram-negative bacteria. The results demonstrated that the MIC for *E. coli* and *S. aureus* were 1 and 0.5 mg/mL, respectively. Moreover, the inhibition zone diameter of 10 mg dilution was 29 mm for *E. coli* and 19 mm for *S. aureus*.[19]

The results of the study by Banoee et al showed that the inhibition zone diameter of the samples were as follows: 17, 12 ± 0.5, 24 ± 0.5, 0, 0, 0, 0.5 ± 8, 0, 0.5 ± 12, 0, 1 ± 12.5, 1.0 ± 12, 0.5 ± 25.0, and 18 ± 1.0 for penicillin-ampicillin, carbenicillin, cefalexin, cefixime, erythromycin, gentamicin, amikacin, tetracycline, ciprofloxacin, clindamycin, nitrofurantoin, nalidixic acid and vancomycin with zinc oxide nanoparticles against *S. aureus*, respectively. While the inhibition zone diameter of these antibiotics against *E. coli* were 0, 0, 0, 0.5 ± 15, 0.5 ± 13, 14, 0, 0.5 ± 11, 1.0 ± 21, 0.5 ± 17, 0.5 ± 17, 0, and 0.5 ± 13 mm, respectively and the concentration of zinc oxide in each disk was 500 µg.[20]

In a similar study by Edalatpanah et al investigating the inhibition zone diameter of acetic acid with different percentages and zinc nano oxide on *S. aureus*, it was revealed that the inhibition zone diameter of 1% acid + 6 mM ZnO, 1% acid + 8 mM ZnO were 8 ± 0.2 and 9 ± 0.2 mm. While the inhibition zone of 2% acid + 6mM ZnO and 2% acid + 8mM ZnO were 10 ± 1 and 11 ± 0.2 mm.[21]

In another study by Mirhendi et al, the diameter of the inhibition zone of zinc nano oxide against *E. coli, S. aureus, Pseudomonas stuzeri*, and *Brevundimonas diminuta* were equal to -22.2 and 0-10 mm.[22]

Conclusion

The results of this study demonstrated good antimicrobial effects of zinc oxide nanoparticles on *S. typhimurium* which can be used to treat infections caused by *S. typhimurium.*

Conflict of Interests

None.

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