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Synthesis of ZnO: Ag nanoparticle and Evaluation its Antimicrobial Activity against Common Isolated Bacterial Pathogens from Dairy Products

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Abstract

The most important feature of nanoparticles is to have a higher specific surface area ratio than their counterpart with a larger size. The aim of this study was the synthesis of zinc oxide nanoparticles doped with silver and its antimicrobial effects on common bacterial pathogens isolated from raw dairy products. In this research 45 samples of dairy raw products after dilution of samples, to isolate *Staphylococcus* aureus and *Escherichia coli* was transferred to the Baird Parker Agar and Sorbitol Mac Conkey Agar media respectively and were identified using a series of specific tests. Zinc oxide nano powder doped with silver was synthesized by sol-gel method. Antimicrobial effects of nanoparticles were investigated by well diffusion method. Minimum inhibitory concentration (MIC) of Zinc oxide-doped nano powder and minimum bactericidal concentration (MBC) were determined. The mean diameter zone of the inhibitory growth of strains of *E. coli* PTCC 1399 , *E. coli* (1) and *E. coli* (2) were 22.5, 18.5 and 15.4 mm respectively at a concentration of 50 mg / ml and mean diameter zone of the inhibitory of *S. aureus* PTCC 1189, *S. aureus* (1) and *S. aureus* (2) standard strains were 24.5, 20.4 and 19.5 mm.In this concentration, MIC for *E. coli* PTCC 1399 was 1.75; *E. coli* (isolate 1) and *E. coli* (isolate 2) were 1.55 and 3.13 mg / ml, respectively. According to the present research, it can be concluded that the zinc oxide nanoparticle itself has a good inhibitory effect on two strains of *E. coli* and *Staphylococcus aureus*. One of the ways to improve or change the properties of nanostructures such as zinc oxide is to introduce impurities into its structure. In case of further experiments, this nano particle can be used as preservative.

Key words: Nanoparticle, Zinc oxide, *Staphylococcus*, *Escherichia coli*, Antibiogram

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Introduction

Zinc oxide is an inorganic compound with the chemical formula ZnO and can have three crystal structures. This compound in the form of a white powder is known as white zinc or ziniket and was called tutia in ancient books. Zinc oxide is an amphoteric oxide that is almost insoluble in water but can be dissolved or decomposed in most acids. It decomposes into zinc vapor and oxygen gas at a temperature of 1975 degrees Celsius. Zinc oxide is used in plastic, glass, rubber, paint, glue, and cosmetic and food industries and in cement, ceramics, ointments and batteries (Espitia, et al. 2012). Among the semiconducting metal oxides, only titanium dioxide and zinc oxide are stable in the excited state. The transformation of zinc oxide powder into zinc oxide nanoparticles by increasing the ratio of surface to volume and strengthening the reactivity of zinc oxide causes intensification or emergence of new characteristics in it. The creation of these new properties and characteristics makes it possible to use these nanoparticles in various fields (Salami et al., 2013). Silver has a strong antimicrobial effect against a wide range of microorganisms even in very low concentrations in several decades. The latter has attracted the attention of many researchers. For a long time, silver has been used as an antimicrobial agent and to prevent damage caused by the activity of fungi and bacteria in wound dressings and infectious wounds. The contamination (doping) of zinc oxide and silver nanoparticles has also been carried out during various studies and has brought positive results. Bacteria are considered as the most important causes of foodborne diseases. Many pathogenic bacterial agents naturally exist in the environment and the place where food products are prepared and can contaminate these products. In recent years, the use of inorganic antimicrobial agents in non-food applications has attracted much attention for controlling microbes. Recent studies prove that sulfide nanomaterial's and metal oxides have very good antibacterial properties and antibacterial agents that include these nanomaterials can have very effective antimicrobial properties. Some nanoparticles made of metal oxides are very stable in processing conditions, and in addition to having selective toxicity against bacteria; they have very little toxic effect on human and animal cells, for example, zinc oxide nanoparticle is a non-toxic and biocompatible nanoparticle (Barzegari et al. 2016). *Staphylococcus aureus* is found in 20 to 30% of the human population in a stable form and in 60% of people in an intermittent form. Therefore, people who work in food preparation, processing and distribution centers can transfer bacteria to food if they do not follow the hygiene tips. Staphylococcal food poisoning constitutes the most cases of bacterial food poisoning. This poisoning occurs by eating food containing preformed toxins produced by enterotoxin-producing strains (Asgarpour et al. 2014). coli forms, especially *Escherichia coli*, are considered to be one of the most important causes of gastroenteritis and microbial indicators of water and food contamination, and their presence in drinking water and food indicates the contamination of these substances with other intestinal pathogens. This bacterium is a gram-negative bacillus of the Enterobacteriaceae family, which is commonly found in the intestines of warm-blooded animals. Most strains of *Escherichia coli* are harmless, but some serotypes such as O157:H7 cause food poisoning and diarrhea. In general, *Escherichia coli* is a common cause of food poisoning (Tavakoli et al. 2013 and Safarpour Dehkordi et al. 2013). The aim of this study is the synthesis of zinc oxide nanoparticles contaminated with silver and its antimicrobial effects on common bacterial pathogens isolated from raw products. Dairy and comparing the antimicrobial effects of common antibiotics with silver-doped zinc oxide nanoparticles.

Materials and methods

Isolation of bacteria from dairy products

In this research, 45 samples of raw dairy products such as cream, raw milk and cheese (15 of each) were examined to isolate *S. aureus*. The samples were transferred to Baird Parker Agar and catalase and coagulase tests were used to confirm it. Also, the resulting colonies were cultured on mannitol salt agar mediu and the sensitivity test to novobiocin was used. To isolate *E.*

coli for enrichment purposes, the samples were cultured in 225 ml Trypticase Soy Broth containing 20 mg/l novobiocin and incubated for 24 hours at 37 degrees Celsius. Then for Bacterial isolation of all enriched samples on sorbitol MacConkey agar medium containing 0.5 mg/liter cefixime and 2.5 mg/liter potassium tellurite were cultured and after 24 hours of greenhouse at 37 degrees, sorbitol negative colonies were purified. Also, to check beta-glucuronidase activity, bacteria confirmed as *E. coli* were cultured on a special chrome agar medium and incubated for 24 hours at 37 degrees Celsius. In order to confirm the final confirmation of sorbitol negative and beta glucuronidase negative colonies, from Additional tests were used.

Preparation of *E. coli* PTCC 1399 and *S. aureus* PTCC 1189

These bacteria were obtained from the Scientific and Industrial Research Organization of Iran.

Antibiogram assay

The sensitivity test of the tested bacteria to the selected antibiotics was performed by using Kirby Bauer's Disc Diffusion antibiotic disc in Mueller Hinton agar medium.

Synthesis of zinc oxide nano powder doped with silver by sol-gel method

In this study, the aim was to prepare zinc oxide nanopowder doped with silver in order to use it as an antibacterial substance using the low temperature sol-gel method. For this purpose, parameters such as the concentration and the annealing temperature of the nanopowders were investigated. Precursors of silver nitrate, zinc nitrate and solid soda were measured on an electric scale to the required amount.

XRD analysis

An amount of 0.2 mg of synthesized zinc oxide nanopowder doped with silver was made into a powder and placed in a sample container and exposed to X-rays using a PNA analyiral Xpert diffractometer and a Cuk alpha 180 source with a wavelength of A55/1 was carried out, which system was equipped with a chromator diffractometer light source at a temperature of 25 degrees Celsius.

TEM analysis

Using TEM electron microscope, the affected nanoparticle size was determined, first the samples were vortexed and 50 ml of these samples were placed in a bed containing copper and carbon. Then it was cooled overnight and Different magnifications were analyzed.

Investigating the antimicrobial properties of nanoparticles using the well diffusion method

In the well diffusion method, the microbial suspension equivalent to half of McFarland was cultured on the surface of Mueller Hinton agar medium in a uniform (table) manner, then using a Pasteur pipette, wells with a diameter of 7 mm were dug and the amount of 20 A microliter of Muller Hinton's culture medium was poured into the bottom of the well and 50 microliters of each of the different concentrations of the extracts were added to each of the wells (7 wells, nanoparticle concentrations were added to wells A to 6, and to the seventh well Sterile distilled water and dimethyl sulfoxide were added as controls (after 2 to 3 hours, it was placed in the refrigerator and then incubated for 24 to 48 hours at 37 degrees Celsius). This step was repeated 3 times. Then, by measuring the diameter of non-growth halos around each well, the sensitivity or resistance of the desired bacteria to nanoparticles was determined. Regarding the synergistic effects, equal volumes of the two extracts were found in each of the concentrations and added to the wells. These experiments were repeated three times and the average diameter of non-growth halos was measured. Then the minimum inhibitory concentration (MIC) of zinc oxide nanopowder contaminated with silver and the minimum bactericidal concentration (MBC) were determined.

Results

Checking the proof of existence and checking the structure of zinc oxide nanophotocatalyst doped with silver.

In the examination of the above spectrum, it can be seen that the characteristic peaks of ZnO at 2θ are equal to 31.23 (100), 34.48 (002) and 36.3 (101) and the corresponding peak of Ag, which is slightly similar to the peak of ZnO at 47.98 (103) covered and two other peaks of Ag

at θ2 equal to (103) 39.01 and (200) 42.58 which prove the presence of silver doped with zinc oxide. To check the distribution of particles in the nanocatalyst, the DLS spectrum was taken from the ZnO sample contaminated with silver, and it is shown in Figure 2. The spectrum shows that, based on the statistical distribution, about 35.7% of the accumulated particles have an average diameter of 1354 nm and 64.3% of the accumulated particles have a diameter of 297.2 nm (Chart 2 and Figure 1).

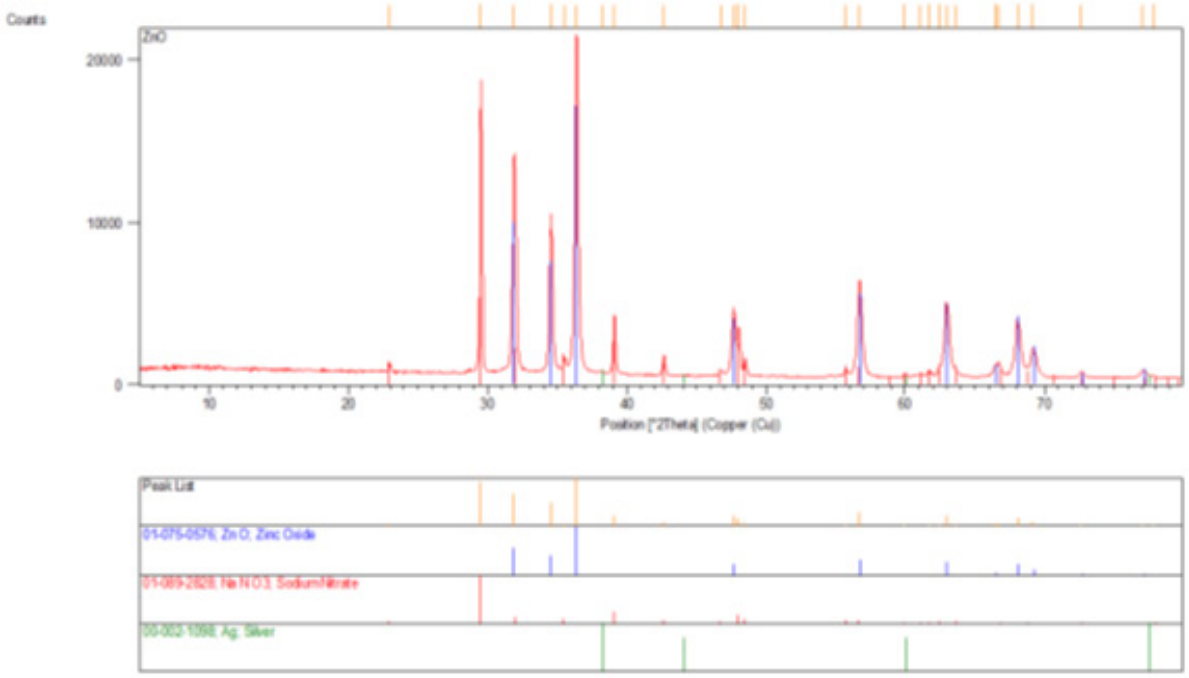

Figure 1. XRD results of zinc oxide nanoparticles doped with silver

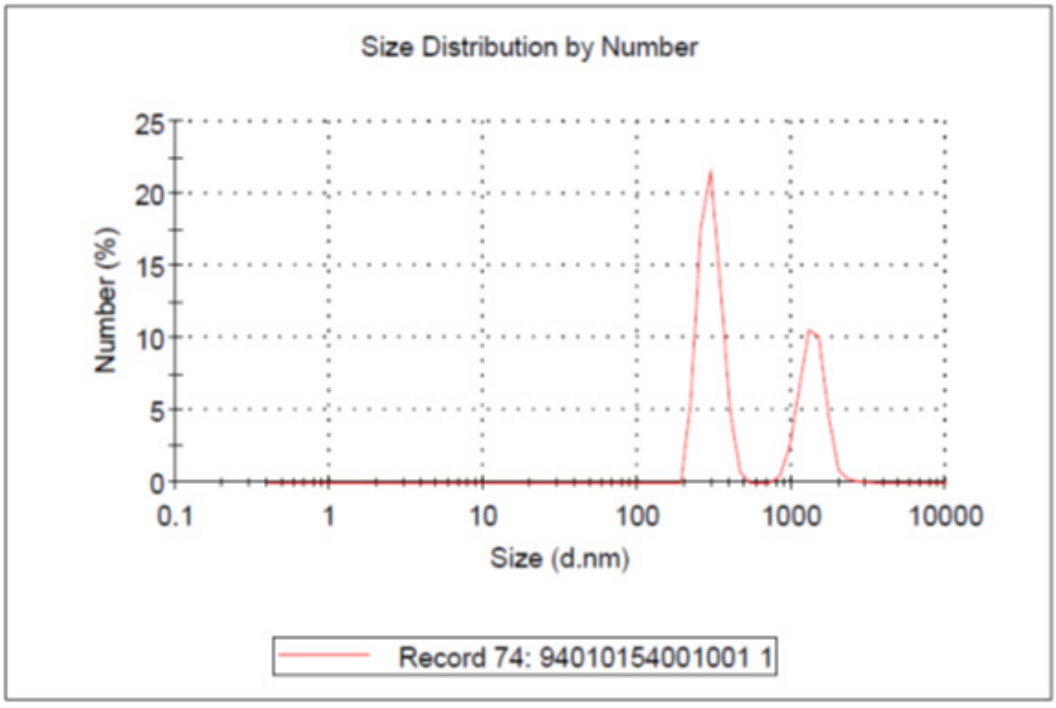

Figure 2. DSL results of zinc oxide nanoparticles doped with silver

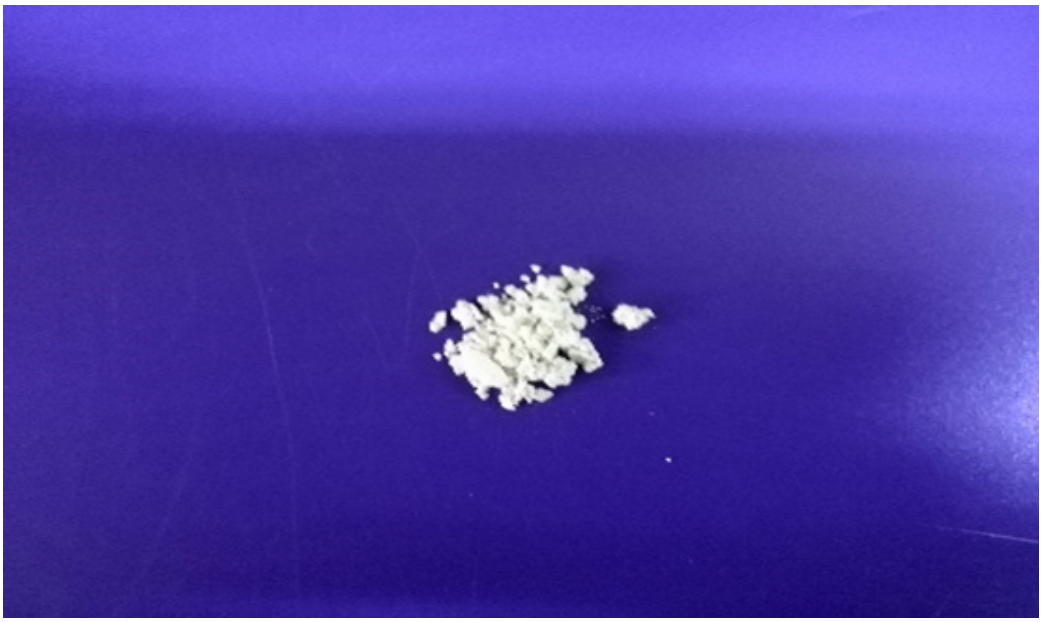

Figure 3. Zinc oxide nanopowder doped with silver

Bacteria isolated from raw dairy products

Out of 45 samples collected from raw dairy products, two samples had *S. aureus* and two samples had *E.coli*.

Resistance and sensitivity of isolated and standard *E. coli* against selected antibiotics

The level of sensitivity and resistance of the investigated microorganisms was determined using the standard antibiogram table. *E. coli* (isolate 1) isolated from raw dairy products was sensitive to the antibiotics cephalexin, ciprofloxacin, amikacin, sulfamethoxazole, nalidixic acid, cefoxime, and nitrofurantoin, but It was resistant to coamoxiclav, cefotaxime, ceftriaxone and ampicillin antibiotics. The level of sensitivity and resistance of *Escherichia coli* (isolate 2) was sensitive to amikacin, cefotaxime, ceftriaxone and nitrofurantoin antibiotics, but resistant to coamoxiclav, ciprofloxacin, sulfamethoxazole and ampicillin antibiotics. So that *S. aureus* (1) is sensitive only to gentamicin and ciprofloxacin, the diameter of which is 24 and 22 mm respectively, but to other antibiotics ofloxacin, penicillin, sulfamethoxazole, cefotaxime, oxacillin and nitrofurantoin. It was resistant. *S. aureus* (2) was only sensitive to ciprofloxacin, the diameter of which was 22 mm, but it was resistant to other antibiotics, ofloxacin, penicillin, sulfamethoxazole, cefotaxime, oxacillin, and nitrofurantoin.

Inhibitory effects of zinc oxide nanoparticles doped with silver

Table 1 shows the average diameter of non-growth halos (mm) of *Escherichia coli* isolated from raw and standard dairy products against silver-doped zinc oxide nanoparticles by the well diffusion method. The largest diameter of non-growth halos was related to the concentration of 50 mg/ml. The average diameter of non-growth halos of standard strains of *E. coli* PTCC 1399 and *E. coli* (1) and *E. coli* (2) were 22.5, 18.5 and 15.4 mm, respectively, at a concentration of 50 mg/ml.

Table 2 shows the average diameter of non-growth halos (mm) of staphylococci isolated from raw and standard dairy products against zinc oxide nanoparticles contaminated with silver by the well diffusion method. The largest diameter of non-growth halos was related to the concentration of 50 mg/ml. The average diameter of non-growth halos of standard strains of *S. aureus* PTCC 1189, *S. aureus* (1) and *S. aureus* (2) were 24.5, 20.4 and 19.5 mm, respectively, at a concentration of 50 mg/ml . The average diameter of non-growth halos of these three bacteria at the concentration of 1.57 mg/ml was 7.2, 5.5 and 3.1 mm, respectively. By reducing the concentration of nanoparticles, its inhibitory effects on bacteria were reduced.

Table 1. The average diameter of non-growth halos (mm) of isolated and standard *E. coli* against different concentrations of silver-doped zinc oxide nanoparticles in the well diffusion method

The concentration of zinc and silver oxide nanoparticles (mg/ml)	*E. coli* PTCC 1399	*E. coli*(1)	*E. coli*(2)
50	22.5	18.5	15.4
25	18.5	15.2	13.5
12.5	17.4	13	11.5
6.25	15.0	11.2	8.2
3.13	11.50	9.5	6.5
1.57	5.20	4.5	·
Blank	·	·	·

Table 2. The average diameter of non-growth halos (mm) of isolated and standard staphylococci against different concentrations of silver-doped zinc oxide nanoparticles in the well diffusion method

The concentration of zinc and silver oxide nanoparticles (mg/ml)	*S. aureus* PTCC 1189	*S. aureus* (1)	*S. aureus* (2)
50	24.5	20.4	19.5
25	21.5	19.2	18.2
12.5	19.5	16.5	14.2
6.25	17.5	14.2	11.5
3.13	15.4	13.5	11.5
1.57	7.2	5.5	3.1
Blank	0	0	0

In this research, the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) of zinc oxide nanoparticles contaminated with silver were determined against selected bacteria. As the MIC for *E. coli* bacteria PTCC 1399 is equal to 57. 1 and *E. coli* (isolate 1) and *E. coli* (isolate 2) were determined as 1.57 and 3.13 mg/ml, respectively, and the MBC for each of the above bacteria was 3.13, 6.25 and 25 mg per milliliter (Table 3).

In this research, the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) of zinc oxide nanoparticles contaminated with silver were determined against selected bacteria. So that the MIC for *S. aureus* PTCC 1189 bacteria is equal to 57 1. and *S.aureus* (isolate 1) and *S.aureus* (isolate 2) were determined as 1.57 and 3.13 mg/ml, respectively, and the MBC for each of the above bacteria was 3.13, 6.25 and 5 It was 12 mg/ml (Table 4).

Table 3. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) of zinc oxide nanoparticles contaminated with silver against isolated and standard E. coli.

Bacterium	*E. coli* PTCC 1399	*E. coli*(isolate 1)	*E. coli*(isolate 2)
MIC	1.57	1.57	3.13
MBC	3.13	6.25	25

Table 4. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) of silver-doped zinc oxide nanoparticles against isolated and standard *S. aureus* strains.

Bacterium	*S. aureus* PTCC 1189	*S. aureus* isolate1)	*S. aureus* (isolate 2)
MIC	1.57	1.57	3.13
MBC	3.13	6.25	12.5

Discussion

Zinc oxide is one of the most important metal oxides used in various industries. Hosseinzadeh and his colleagues investigated the antimicrobial efficiency of 2% and 4% zinc oxide nanoparticle suspension against *E. coli* O157:H7 in 2013, and both concentrations significantly reduced the amount of this bacteria, which The concentration of 4% was significantly higher (Hoseinzadeh, et al. 2012). In the study of Emami and his colleagues, the antimicrobial properties of different concentrations of zinc oxide nanoparticles against *S. aureus* and *E. coli* bacteria were investigated. The results showed that the concentration of 10 mg/ml of zinc oxide nanoparticles showed the greatest inhibitory effect with 22 and 28 mm on *S. aureus* and *E. coli*, respectively (Emami-Karvani, et al. 2011). Azam and his colleagues in the year 2012, investigated the antimicrobial effects of ZnO, CuO and Fe_2O_3 nanoparticles against Gram-positive *S.aureus* and Gram-negative *Escherichia coli* bacteria. The results showed that the ZnO nanoparticle of Derari had the highest antibacterial activity compared to the other two nanoparticles. The minimum lethal concentration (MBC) of zinc oxide nanoparticles was determined to be 18 µg/ml for *Escherichia coli* and 16 µg/ml for *S. aureus* (Azam 2011). In 2014, Barzegari and his colleagues investigated the combined effects of zinc oxide nanoparticles and malic acid on inhibiting the growth of *E. coli* and *S. aureus* bacteria and observed that suspension treatment (0, 1,3,5,8)mM of zinc oxide nanoparticles In malic acid, it shows a significant inhibitory effect on the growth of *Escherichia coli* and *S. aureus* during 42 hours of incubation. Also, the concentration of 5 and 8 mM zinc oxide nanoparticles together with malic acid showed the greatest inhibitory effect on *Escherichia coli* and S.aureus, 11 and 34.5 mm, respectively (Barzegari Firouzabadi et al. 2016). In 2012, Wang et al investigated the antimicrobial effects of zinc oxide on *Escherichia coli* K88. This nanoparticle showed strong inhibitory effects. This inhibition was directly related to the increase in nanoparticle concentration. MIC and MBC were 0.1 and 0.8 µg/ml, respectively (Wang, et al. 2012). According to the research done and the current research, it can be concluded that the zinc oxide nanoparticle itself has a good inhibitory effect. Zinc is present in two strains of *Escherichia coli* and *S. aureus*, and *S. aureus* seems to be more sensitive to this nanoparticle than *Escherichia coli*. One of the ways to improve or change the properties of nanostructures such as zinc oxide is to introduce impurities into its structure. Silver has attracted the attention of many researchers due to its strong antimicrobial effect against a wide range of microorganisms even at very low concentrations in the last few decades. For a long time, silver has been used as an antimicrobial agent and to prevent damage caused by the activity of fungi and bacteria in wound dressings and infectious wounds. The contamination (doping) of zinc oxide and silver nanoparticles has also been carried out in various studies and has brought positive results. In the study of Wang and his colleagues in 2017, the antimicrobial activity of Ag/ZnO on *S. mutans* was investigated. The results showed that Ag/ZnO has higher antimicrobial effects compared to ZnO (Wang et al. 2017). In the study of Matai and his colleagues in 2013, the antimicrobial properties of Ag-ZnO nanoparticles against *S. aureus* and antibiotic-resistant strains of *Escherichia coli* were investigated, and the results showed that the minimum inhibitory concentration for resistant *Escherichia coli* and *S. aureus* was The order of 550 and 60

micrograms per milliliter of nanoparticles was observed (Matai et al. 2014). Of course, this value in the study of Hu and his colleagues (2008) for *E. coli* and *S. aureus* was 600 and 400 micrograms per milliliter of Ag nanoparticles, respectively. -ZnO was observed (Hu, et al. 2016). Research by Hu and his colleagues (2016) showed that Ag/ZnO with 5% mol of silver concentration had an inhibitory effect against *Staphylococcus* aureus, *E. coli* with the inhibition rate of 21.7 and 18.4 mm, respectively (Hu, et al. 2016). This value was similar to the average inhibition of the present study, which was 19.95 mm for two isolates of *S. aureus* and 16.95 mm for *E.coli*. In the review of Venkatasubramanian and Sundaraj in 2014, the antimicrobial activity of Ag/ZnO against *E. coli* bacteria was investigated and it showed that the use of 0.025% concentration of zinc oxide and 7.5% silver has high antimicrobial properties. This value was higher than when only zinc oxide nanoparticles were used (Venkatasubramanian, Sundaraj 2014). As can be seen in the current and previous researches, the level of resistance and sensitivity of the strains is different and it can change according to the type of food, geographical location, preparation conditions, etc. With the increase of antibiotic resistance of bacteria, the use of alternative treatments such as the use of nanoparticles can be very appropriate.

Conclusion

Since the antimicrobial effect of zinc oxide nanoparticle is specific for each strain, special studies are needed for the application of zinc oxide nanoparticle to control bacteria and other elements are contaminated with it. Also, the antimicrobial effects of this pollutant in in vivo conditions have also been studied. be checked As can be seen in the current and previous researches, the level of resistance and sensitivity of the strains is different and it can change according to the type of food, geographical location, preparation conditions, etc. With the increase in antibiotic resistance of bacteria, the use of alternative treatments such as the use of nanoparticles can be very suitable. As can be seen in the present research and the previous research, the resistance and sensitivity of the strains are different and according to the type of food, geographic location and conditions of preparation, etc. can vary. With the increase of antibiotic resistance of bacteria, the use of alternative treatments such as the use of nanoparticles can be very appropriate.

Conflicted of Interest

No conflicts of interest

References

Tavakoli H, Sarshar M, Ranjbar R, Sadr Mumtaz M and Rafati H (1992). Contamination with *Escherichia coli* O157:H7 in selected foods in some restaurants in Tehran using chromogen culture media. Infectious and tropical diseases quarterly affiliated to the Association of Infectious and Tropical Diseases Specialists, 63, 61 – 66.

Saliani M, Jalal R and Kafshdare Goharshadi E (2015). Effects of pH and Temperature on Antibacterial Activity of Zinc Oxide Nanofluid against *Escherichia coli* O157: H7 and *Staphylococcus* aureus. Jundishapur J Microbiol. 15, 8(2), 17115. doi: 10.5812/jjm.

Safarpour Dehkordi, F. Rahimi, A. Ghobadi, M and Yahaghi, A (2013). *Escherichia coli* producing Shiga toxin in sheep cheeses. Journal of infectious and tropical diseases 66, 52-52.

Asgarpour D and Dighmi, H (2014). The role of *Staphylococcus aureus* enterotoxins in staphylococcal food poisoning: a systematic review article. Laboratory and diagnosis quarterly, 28, 63-73.

Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A (2012). Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomedicine, 7:6003-9.

Barzegari Firouzabadi F, Marzban Z, Khaleghizadeh S, Daneshmand F and Mirhosseini M (2016). Combined effects of zinc oxide nanoparticle and malic acid to inhibit *Escherichia coli* and *Staphylococcus* aureus. Iran J Med Microbiol, 10 (5), 52-59.

Emami-Karvani, Z and Chehrazi, P (2012). Antibacterial activity of ZnO nanoparticle on Gram-positive and Gram-negative bacteria. African Journal of Microbiology Research. 5(10), 5897.

Faramarzi T, Jonidi Jafari A, Dehghani S, Mirzabeygi M, Naseh M and Rahbar Arasteh H(2012) A survey on Bacterial Contamination of Food Supply in the West of Tehran. JABS, 2 (1), 11-18.

Ghosh T, Das A, Jena B and Pradhan C (2015). Antimicrobial effect of silver zinc oxide (Ag-ZnO) nanocomposite particles. Frontiers in Life Science, 8. 10, 1080.

Gündoğan N, Citak S and Turan E (2016) .Slime

production, DNase activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurised milk and ice cream samples. Food Control, 17(5), 389-92.

Hu YW, He HR, Kong X, Ma YM (2016) .Synthesis and Antibacterial Activities of Ag/ZnO Nanoparticles. KEM, 697,714–7.

Matai I, Sachdev A, Dubey P, Kumar S U, Bhushan B and Gopinath P (2014). Antibacterial activity and mechanism of Ag–ZnO nanocomposite on *S. aureus* and GFP-expressing antibiotic resistant *E. coli*. Colloids and Surfaces B: Biointerfaces, 115, 359-367.

Venkatasubramanian K & Sundaraj S (2014). Antibacterial activity of Zinc Oxide and Ag doped Zinc Oxide Nanoparticles against *E. coli*. Chem Sci Rev Lett, 3, 40-44.

Wang C, Lian-Long L, Ai-Ting Z, Peng X, Jian-Jun L and Xiao-Ting Z (2012). Antibacterial effects of zinc oxide nanoparticles on *Escherichia coli* K88. African Journal of Biotechnology 11(44), 10248-10254.

Wang S, Wu J, Yang H, Liu X, Huang Q and Lu Z(2017). Antibacterial activity and mechanism of Ag/ZnO nanocomposite against anaerobic oral pathogen *Streptococcus mutans*. J Mater Sci Mater MedJan; 28(1), 23.