

RESEARCH ARTICLE

Evaluation of the Inhibitory Effects of Magnesium Oxide and Copper Oxide nanoparticles on Biofilm Formation of Some Foodborne Bacterial Pathogens

Praisa Hashemifard Dehkordi^{1*}, Hamdollah Moshtaghi Brujeni¹ and Maryam Abbasvali^{2,3}

¹Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

²Department of Food Hygiene and Quality Control, Shiraz University of Medical Sciences, Shiraz, Iran

***Corresponding author:** Praisa Hashemifard Dehkordi, Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran, Tel: +989133810896, E-mail address: phashemifard@yahoo.com

Citation: Praisa Hashemifard Dehkordi, Hamdollah Moshtaghi Brujeni, Maryam Abbasvali (2022) Evaluation of the Inhibitory Effects of Magnesium Oxide and Copper Oxide nanoparticles on Biofilm Formation of Some Foodborne Bacterial Pathogens. J Nanosci Nanotechnol Appl 6: 104

Abstract

One of the most important factors in food industry is the formation of microbial biofilm which can be the potential source of food products contamination with food spoilage and foodborne pathogenic bacteria. Nanotechnology is considered as a promising solution to produce and develop such novel antimicrobial substances. The potential effects of nanostructured metal oxides on the reduction of such contaminants are well established. The goal of this study was to see how well magnesium oxide (MgO) and copper oxide (CuO) nanoparticles (NPs) inhibited growth and biofilm formation of four common foodborne bacterial pathogens. Copper oxide (CuO) nanoparticles with a Purity of: 99% (metal base) and Average size of copper nanoparticles: 10-30 nm, Specific surface area of copper nanoparticles: 35 square meters/gram, Color of copper nanoparticles: black brown, The apparent density of copper nanoparticles: 0.35-0.15 grams per cubic centimeter, Actual density of copper nanoparticles: 8.94 grams per cubic centimeter and Manufacturing method of copper nanoparticles: It is made by electrochemical method. Magnesium oxide (MgO) NPs with a Purity of: 99.9%, Size of magnesium nano oxide particles: 10-30 nm, Specific surface area of nanoparticles: 85-120 square meters per gram, Color of magnesium oxide nano powder: white, Morphology of magnesium oxide nanoparticles: multifaceted, Bulk density of nano powder: 0.68 grams per cubic centimeter and the real (particle) density of nanoparticles: 3.58 grams per cubic meter.

This study was completed in the year 2020. Resazurin reduction and micro-dilution procedures were used to assess the minimum inhibitory concentration (MIC) of magnesium oxide and copper oxide nanoparticles for *Escherichia coli* O157: H7 (ATCC 35218), *Listeria monocytogenes* (ATCC 19118), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 14028).

Magnesium oxide nanoparticles had MICs of 2, 2, 2 and 4 mg/ml, while copper oxide nanoparticles had MICs of 1, 0.16, 0.5 and 0.25 mg/ml against *E. coli*, *L. monocytogenes*, *Staph. aureus* and *P.aeruginosa* respectively. At the MIC, the magnesium oxide nanoparticles inhibited biofilm formation of *E. coli*, *L. monocytogenes*, *Staph. aureus* and *P.aeruginosa* by 89.9, 96.6, 98.3 and 98.3 percent and the copper oxide nanoparticles inhibited biofilm formation 88, 97.7, 93.6 and 98.7 percent, respectively. Both compounds had inhibitory effects on *E. coli*, *L. monocytogenes*, *Staph. aureus* and *P.aeruginosa* according to our findings. Even at sub-MICs, NPs were found to be able to prevent biofilm development. MgO and CuO nanoparticles can be utilized as a cleaning agent for surfaces to avoid the formation of foodborne bacterial biofilms, which is important for public health.

Keywords: biofilm, magnesium oxide nanoparticle, copper oxide nanoparticle, Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus, Pseudomonas aeruginosa

Introduction

Safety in food production and storage is an important public health concern all over the world. Despite the increasing growth of the food industry and the observance of health principles in the production and processing of food, microbial contamination is known as the main source of frequent outbreaks of food-borne diseases. Therefore, finding new antimicrobial compounds to ensure the health of food and increase their shelf life seems absolutely necessary [15]. Biofilm is a wide-range life form of bacteria that consists of the association of microorganism's cultures and extracellular polymer matrix (EPM), a complex biochemical mixture of polysaccharides, proteins, glycopeptides, nucleic acids, and lipids. In biofilm formation, there are three main stages: adhesion, colonization, and maturation. From the mature biofilm, the plankton microorganisms are isolated or dispersed into the environment. Thus, biofilm is a complex three-dimensional biological structure with a higher microbial life organization, in many respects, similar to a multicellular organism [11]. The production of biofilms in bacteria is a technique for germs to preserve and thrive in harsh environments. Bacteria can be protected from host attack and defense by utilizing this crucial process. Antibiotics, chemical poisons, environmental challenges, particularly drought stress, and abrupt changes in acidity and ambient temperature are all very resistant to bacteria based on biofilm [21]. Biofilm can grow on a variety of surfaces, including natural water systems, body tissues, medical and industrial devices, drinking water systems, and nearly any other surface. Within the matrix, biofilms can be benign, pathogenic, or toxin-producing [11]. The major purpose of biofilm is to shield bacteria within it from harmful physical, chemical, and biological elements in the environment, such as temperature, dryness, UV radiation, biocides, humoral, and cellular immune factors [34]. Biofilms play an important role in the environment, industry, and medicine. [32]. Maintaining food safety and quality has long been a concern for food industry specialists and health regulators, and ignoring or undervaluing it can result in irreparable harm to society. Diseases caused by ingesting tainted food are a serious problem today all over the world. One of the key goals of food producers is to create healthy, safe, and high-quality food in order to ensure food safety in society. In addition to endangering the consumer's health, rotting food costs the manufacturer money [7]. Because the creation of microbial biofilm on surfaces has such a substantial impact on food quality and safety in contact with food, the production of microbial biofilm on surfaces might be considered the most serious problem in the food industry [4]. Biofilm formation is one of the primary and most serious challenges in the food industry, notably in the beer, dairy, red meat, poultry, and fish industries, as there have been several cases of food contamination as a result of contact with biofilm contaminated surfaces [2]. Bacterial pathogens, which are particularly important in food safety, are among the microorganisms that create biofilms in food manufacturing environments. These bacteria can form biofilms on a variety of artificial substrates used in the food business, including stainless steel, polyethylene, wood, glass, polypropylene, rubber, and others [10]. Food-borne pathogens found in biofilms on food matrixes or industrial equipment can cause intoxications or infections. Biofilm present in food processing plants, for example, can secrete toxins. They can then contaminate a food matrix, causing individual or many intoxications (in the case of an outbreak) [8]. Microorganisms can easily be spread when people come into contact with contaminated surfaces. Because the surfaces of food-contact machinery in the food industries are often composed of stainless steel, frequent use causes scratches and roughness, and some food remains on these surfaces, increasing bacteria's potential. It develops and produces a biofilm at the junction that is resistant to cleaning and disinfecting treatments. Microbial contamination in the product is caused by the growth of these microorganisms on equipment and food, which diminishes the shelf life of the product and raises the occurrence of foodborne disorders. Another issue with biofilms is that they can build in locations like heat exchangers in food processing equipment, reducing heat transfer efficiency. Furthermore, some of the microorganisms in the biofilm cause metal surfaces to corrode. Biofilm cells are more resistant to bactericidal and disinfection treatments than free cells, making it more difficult for bacteria to form biofilms on surfaces, which makes washing and killing them more difficult [12]. It's critical for the food business to figure out how to prevent biofilms from forming on surfaces that come into touch with food, as well as how to get rid of them, and it's also vital for society's overall health. In the food business, effective cleaning and disinfection of equipment and production lines are two critical challenges in biofilm

control. Antimicrobial substances that prevent the formation of biofilms of foodborne pathogenic bacteria are critical in this regard. As bacteria become increasingly resistant to antibiotics, a new strategy for managing biofilms is clearly required. As a result, identifying new antibacterial chemicals is critical for ensuring food safety and extending shelf life [20]. Recent advances in nanotechnology, particularly the ability to create nanoparticles in a variety of shapes and sizes, have led to the development of antimicrobial treatments. A higher surface-to-volume ratio makes nanomaterials more physiologically active when compared to larger particles of the same chemical components [7]. The surface activity of the material increases dramatically when particle size is reduced to the nanoscale level, and the ratio of the material's reaction with the environment increases as a result of the increase in surface active sites [13]. For the treatment of bacterial biofilms, nanoparticles (NPs) are thought to be a viable approach. It's because antibiotic resistance mechanisms don't work against NPs [44]. The interaction between NPs and biofilms can be broken down into three stages: (1) NPs transfer near the biofilm, (2) NPs attachment to the biofilm surface, and (3) biofilm movement. Many elements influence the implementation of each stage, including NPs physicochemical properties, extracellular polymeric matrix (EPM) and the environment (Tong et al. 2010). The nanoparticle has emerged as a novel drug molecule in last decade and has been used in various industrial fields like cosmetics, healthcare, agricultural, pharmaceuticals due to their high optical, electronic, medicinal properties. Use of nanoparticles as an antibacterial agent remain in current studies with metal nanoparticles like silver, gold, copper, iron and metal oxide nanoparticles like zinc oxide, copper oxide, titanium oxide and iron oxide nanoparticles. The high anti-bacterial activity of nanoparticles is due to their large surface area to volume ratio which allows binding of a large number of ligands on nanoparticle surface and hence, its complexation with receptors present on the bacterial surface. Of all-metal oxide nanoparticles, magnesium oxide (MgO) nanoparticles have good activity for use as an antibacterial. Leung et al. [6] explained that the antibacterial activity of MgO nanoparticles can be observed based on the absence of reactive oxygen species (ROS). The mechanism of antibacterial activity that occurs is possible by destruction of cell membranes. The activities of MgO against bacteria have been studied by several researchers previously. Mirhosseini and Afzali [14] studied the antibacterial activity of MgO suspension against *Escherichia coli* (*E. coli*) in milk. Scanning using an electron microscope was performed to characterize the morphological changes of the *E. coli* after antibacterial treatment. The results obtained indicate that the presence of MgO combined with pressure can damage cell membranes, resulting in a leakage of the cell contents, and eventually, the bacterial cell die. Copper/cupric oxide has excellent physicochemical features, such as antibacterial, antioxidant, low-cost and is non-toxic, which make it an affordable and valuable material with enormous utility and it is also a very effective semiconductor material. Copper and copper oxide nanoparticles have promising usages as water remediation agents, sensors, photocatalysts, etc. Many biological synthesis approaches have been used to produce copper oxide nanoparticles, but green approaches are considered to exhibit better antibacterial and electrochemical properties. It has been observed that green synthesized copper oxide nanoparticles exhibit potential antibacterial properties when observed against various bacterial strains. Similarly, they have also shown good antioxidant, electrochemical, optical and catalytic properties. These properties have led to copper oxide nanoparticles attracting interest for many potential applications, like antimicrobial activity, electrochemical sensing, etc. The US Food and Drug Administration (FDA) considers magnesium oxide nanoparticles (MgO NPs) to be a safe drug for human health [43]. Gram-positive and Gram-negative bacteria, spores, and viruses are all susceptible to MgO NPs [31]. The generation of alkalinity species such as superoxide and electrostatic adsorption of nanoparticles onto the bacterial cell wall could be the sources of its antibacterial activity [25]. Copper oxide nanoparticles (CuO NPs) is the most basic of the Cu compounds, revealing a wide range of potential physical features while being significantly less expensive than silver oxide. It's simple to combine with polymers to provide composites specific physico-chemical properties. CuO NPs have a dose-dependent antibacterial effect due to their high surface areas and distinctive crystalline morphologies (Huang et al. 2005). CuO NPs prevent the formation of biofilms and aid in the elimination of those that have already formed. Copper ions are poisonous to plankton and biofilm cells; hence this is the fundamental reason (Agarwal et al. 2014).

Materials and Methods

MgO NPs and CuO NPs (US Research Nanomaterials, Inc.) with a purity of 99 percent and a particle size of 10-30 nm were obtained from the Iranian Nanomaterials Pioneers Company (Mashhad, Iran) for this investigation, which was conducted in the year 2020.

Preparation of magnesium oxide and copper oxide nanoparticles suspension

Magnesium oxide and copper oxide nanoparticles with 99% purity and particle size of 10-30 nm were purchased. The shape and size of nanoparticles were determined by using a scanning electron microscope (SEM) and Transmission electron microscope (TEM) (Figure 1 and 2) and X-ray Diffraction Analysis (XRD) checked and determined. To prepare the stock solution, the nanoparticles powder was first suspended in Tryptic Soy Broth, Merck. Then the suspension was autoclaved at 121 degrees Celsius for 15 minutes and homogenized in an ultrasonic bath (Gran XB6, UK) for 30 minutes.

The Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord University, provided *E. coli* (ATCC 35218), *Listeria monocytogenes* (ATCC 19118), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 14028) standard strains for this study. Bacterial strains were maintained in Trypticase soy broth (TSB) with 30% glycerol as a growth medium and kept at -80°C . The strains were defrosted at room temperature before being cultivated in TSB and incubated at 37°C for 24 h, followed by another 24 h at 37°C on specific culture media such as McConkiey agar and Palkam agar. A pure colony was taken from each bacterium the day before the experiment and put to the TSB medium. The appropriate dilution of the bacterial suspension was generated using sterile physiological serum on the day of the experiment.

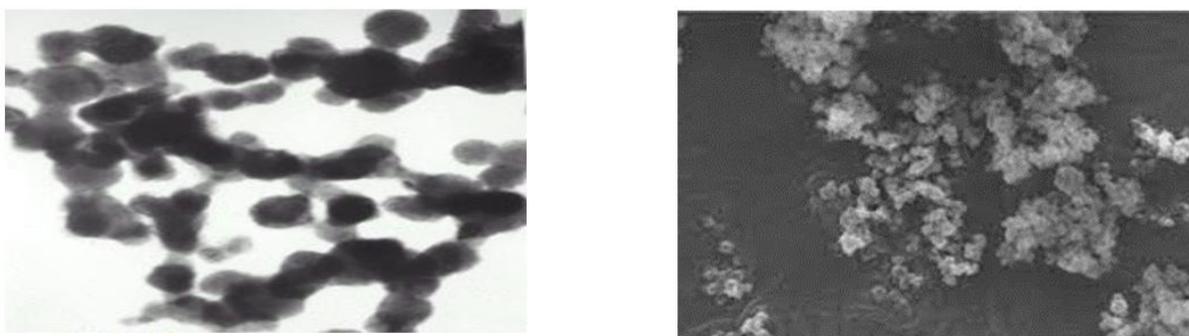


Figure 1: Scanning Electron Microscope (SEM) Magnesium Nan oxide (Right) Copper Nano Oxide (Left)

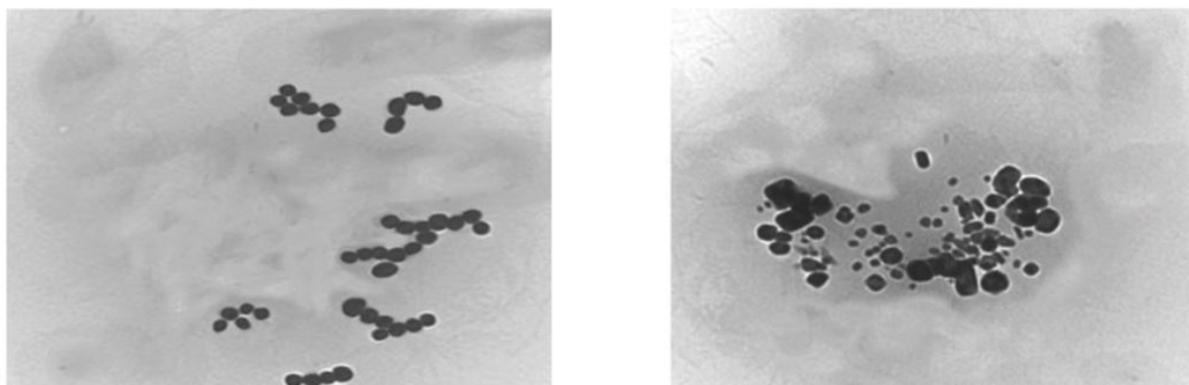


Figure 2: Transmission Electron Microscope (TEM) Magnesium Nan oxide (Right) Copper Nano Oxide (Left)

Nanoparticles Minimum Inhibitory Concentration Assay Using Resazurin Color Detection method

A sterile 96-well microplate was utilized according to Sarker's approach [37]. Initially, 300 mg of the fully uniformed resazurin color reagent was dissolved in 40 ml of distilled water. It was then transferred to a sterile tube after being sterilized using a syringe filter.

80 mg of magnesium oxide nanoparticle powder was combined with TSB medium to form a 10 ml solution to make a stock of magnesium nanoparticles with an 8 mg/ml concentration. The suspension was sonicated for 30 minutes before being autoclaved at 121°C for 15 minutes to homogenies it. To prepare a 10 ml suspension, 40 mg of copper oxide nanoparticle powder was combined with TSB medium. To homogenies the suspension, it was sonicated for 30 minutes before being autoclaved at 121°C for 15 minutes.

One milliliter of bacteria was cultured for 24 h at 37°C in 9 ml of normal sterile saline, which was then diluted repeatedly to reach a concentration of 10^7 CFU/ml. To determine the MIC against two bacteria species, a twofold dilution with a volume of 100 μ l was made in a 96-well microplate with a concentration of 8 and 4 mg/ml magnesium oxide and copper oxide nanoparticles, respectively, in TSB culture media. A well was filled with 100 nanoparticles. Then, in each well, 10 μ l of 10^7 CFU/ml bacterial suspension, 30 μ l of resazurin solution, and 60 μ l of TSB medium were added. Finally, the magnesium nanoparticle concentrations in each well were 4, 2, 1, and 0.5 mg/ml, while the copper nanoparticle concentrations in each well were 2, 1, 0.5, and 0.25 mg/ml, and the bacterial concentration was 10^5 CFU. Culture medium control (170 μ l of TSB and 30 μ l of resazurin solution, and 10 μ l of bacterial suspension), positive control (160 μ l of TSB and 30 μ l of resazurin solution, and 10 μ l of bacterial suspension), and negative control (100 μ l of stock nanoparticles, 70 μ l of culture medium, and 30 μ l of resazurin solution, and 10 μ l of bacterial suspension) were considered. Three replications were performed for each bacterium and control.

The results were checked after the microplates had been incubated in the incubator for 24 h at 37°C. The color of resazurin changed from purple to colorless, which was seen and compared to the control color of nanoparticles and each bacterium's control. Finally, the MIC was established for each bacterium as the lowest concentration of nanoparticles that inhibited the bacterium's growth.

Biofilm test

The potential of the examined microorganisms to build biofilm was tested using 96-well microplates with a flat bottom, as described by Tendolkar et al [42]. The bacteria in the TSB medium were cultured for 24 h at 37°C for this purpose. A total of 8 wells were designated to each bacterium in each row of microplates with 12 wells, with the remaining four wells being utilized as negative controls (200 μ l of TSB). Each well received 100 μ l of diluted suspension of each bacterium at a concentration of 10^6 CFU/ml, along with 100 μ l of TSB medium, and the microplates were placed at 37°C for 24 h.

After this time, the microplates were returned to empty the culture media in each well, then washed three times with sterile phosphate buffer to eliminate bacteria that had not formed biofilms. The microplates were then dried for one-hour upside down at room temperature. Each well was filled with 200 μ l of 0.2 percent violet crystal solution, and the microplate was immobilized at 37°C for 15 minutes without any movement. The dye then poured out into the wells, and the microplates dried at room temperature after being washed with sterile phosphate buffer. To remove the dye bonded with the biofilm-forming bacteria, 200 μ l of a 30% acetic acid solution was put into each well.

With a microplate reader, the optical absorbance of this solution was measured at 595 nm, and the ability to form a biofilm was assessed using the method described by Stefanovic et al [40]. The mean light absorption of negative controls was summed by three times the deviation from the criterion of light absorption of negative controls. The resulting number was called the Optical Density cut-off value (ODC). Based on that, bacteria were divided into four categories in terms of biofilm formation:

A: Bacteria without biofilm; optical absorption is less and equivalent to ODC in this category.

B: bacteria that produce weak biofilm; optical absorption was higher than ODC but less than or equal to twice that of ODC in this class.

C: bacteria that formed a moderate amount of biofilm and had an optical absorption greater than twice that of ODC but less than or equal to four times that of ODC.

D: bacteria with a strong biofilm; the optical absorption of this class was more than four times that of ODC.

Nanoparticles' ability to prevent biofilm development

Bacteria in TSB medium were cultured for 24 h at 37°C, after that, serial dilution was used to create stock bacteria at a concentration of 10⁶ CFU/ml. Each microplate contained two bacteria, and for each stock bacterium, nanoparticles with a 4-fold concentration (4MIC) were made. In each row, 100 µl of TSB medium was poured into the wells, followed by 100 µl of 4MIC nanoparticle stock in the first well, and dilution was done in a double series. Finally, the nanoparticle concentrations in the wells were 2 MIC, MIC, 1/2MIC, 1/4MIC, and 1/8 MIC.

100 µl of stock bacteria were put into each well at a concentration of 10⁶ CFU/ml. These processes were repeated three times for each bacterium (3 replications). The nanoparticle control consisted of a row of microplates. In the wells, only the culture media and various quantities of nanoparticles were put. The first and second four wells in a row, each containing 100 µl of bacteria with a concentration of 10⁶ CFU/ml plus 100 µl of culture media, were used to control the growth of the first and second bacteria, respectively. Only 200 microliters of culture medium were placed into the last four wells of this row, which were also used as control for the culture medium. The microplate was kept at 37°C for 24 h. The culture media in each well was drained after this time, and the wells were washed three times with sterile phosphate buffer. The microplates were then inverted for 1 hour at room temperature to dry. Each well was filled with 200 µl of 0.2 percent crystal violet solution, and the microplate was immobilized at 37°C for 15 minutes without moving. The dye was then removed from the wells and the microplates were washed with sterile phosphate buffer before being dried at room temperature. To remove the dye bonded with the biofilm-forming bacteria, 200 µl of a 30% acetic acid solution was applied to each well. A microplate reader was used to measure the light absorption of this solution at 595 nm wavelengths. Finally, in the presence of varied doses of nanoparticles, the percentage of inhibition of biofilm formation of each bacterium was calculated using the formula [30]:

$$\text{Percentage of biofilmformation inhibition} = \frac{(\text{Absorbance of sample without bacteria} - \text{Absorbance of sample containing bacteria}) - \text{Absorbance of growth control of each bacterium}}{\text{Absorbance of growth control of each bacterium}}$$

Statistical analysis

The tests were performed three times in this study, and the data was analyzed using the analysis of variance (ANOVA) method in the SPSS programmed. Duncan's multiple range test was used to compare means at the probability level ($P < 0.05$). The effect of nanoparticles on the suppression of biofilm development by gram-positive and gram-negative bacteria was compared using the student's t test.

Result

Determination of the MIC of nanoparticles using the resazurin color reagent

The MIC (mg/ml) of magnesium oxide and copper oxide nanoparticles for the examined microorganisms are shown in Table 1. Magnesium oxide nanoparticles had MICs of 2, 2, 2 and 4 mg/ml, while copper oxide nanoparticles had MICs of 1, 0.16, 0.5 and 0.25 mg/ml against *E. coli*, *L. monocytogenes*, *Staph. aureus* and *Paeruginosa* respectively.

The name of the bacteria	MIC of magnesium oxide NPs	MIC of copper oxide NPs
<i>Listeria monocytogenes</i>	2	1
<i>Escherichia coli</i> O157:H7	2	0.16
<i>Staphylococcus aureus</i>	2	0.5
<i>Pseudomonas aeruginosa</i>	4	0.25

Table 1: The minimum inhibitory concentration (MIC) (mg/ml) of magnesium oxide and copper oxide nanoparticles (NPs) in the tested bacteria

Evaluating the biofilm formation ability of the studied microorganisms

The biofilm formation ability of the studied microorganisms is shown in Table 2. The ability to form biofilm in the three bacteria *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* was statistically the same, and no significant difference ($P < 0.05$) was observed between these three groups. The ability to form biofilm in *Pseudomonas aeruginosa* bacterium was higher than all groups, so it has a significant difference ($P < 0.05$) with the previous three groups.

The name of the bacteria	The power of biofilm formation
<i>Listeria monocytogenes</i>	Weak
<i>Escherichia coli</i> O157:H7	Weak
<i>Staphylococcus aureus</i>	Weak
<i>Pseudomonas aeruginosa</i>	Medium

Table 2: Biofilm formation ability of bacteria based on ODc classification (Optical Density cut-off value)

Determination of the percentage of biofilm formation

Tables 3 and 4 show the percentage of bacterial biofilm formation that is suppressed in the presence of different concentrations of magnesium oxide and copper oxide nanoparticles. As seen in the tables, nanoparticles at sub-MIC concentrations were also able to prevent biofilm formation. When compared to the concentrations of MIC and 2MIC, the capacity to suppress biofilm formation of 1/2MIC nanoparticles did not show any statistically significant difference ($P > 0.05$).

Bacteria	4MIC	2MIC	MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
<i>Listeria monocytogenes</i>	99.7	99.3	96.6	94.6	87.7	74.8	71.5
<i>E. coli</i> O157:H7	99.3	99	89.9	86.1	80.3	74.9	67.5
<i>Staphylococcus aureus</i>	99	98.7	98.3	88.7	81	63.6	51.4
<i>P. aeruginosa</i>	99.6	99	98.3	75.7	53	47.1	37.9

Table 3: Mean percentage inhibition of biofilm formation of each bacterium in the presence of different concentrations of magnesium oxide nanoparticles

Bacteria	4MIC	2MIC	MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
<i>Listeria monocytogenes</i>	98.7	98.5	98	97.8	97	86	85.2
<i>Escherichia coli</i> O157:H7	99.3	99.0	93.6	78	70.5	70	67
<i>Staphylococcus aureus</i>	99.7	99.3	88	69.7	66.8	61.7	61.7
<i>P. aeruginosa</i>	99.3	98.7	97.7	96.6	76.1	59.1	28.5

Table 4: Mean percentage inhibition of biofilm formation of each bacterium in the presence of different concentrations of copper oxide nanoparticles.

In the presence of different concentrations of magnesium oxide and copper oxide nanoparticles, Figure 3 compares the mean percentage of inhibition of *Escherichia coli* biofilm formation. Magnesium oxide nanoparticles have a higher inhibitory effect on *Escherichia coli* biofilm development. Therefore, the inhibitory effect of the two nanoparticles differs significantly ($P < 0.05$) in 1/2 and 1/4 MIC and the difference in 1/4 MIC reached 10%. Following the decrease in nanoparticle concentration, the difference between the inhibition percentages of the two nanoparticles in 1/8 and 1/16 MIC decreased until there was no significant difference ($P > 0.05$) between them.

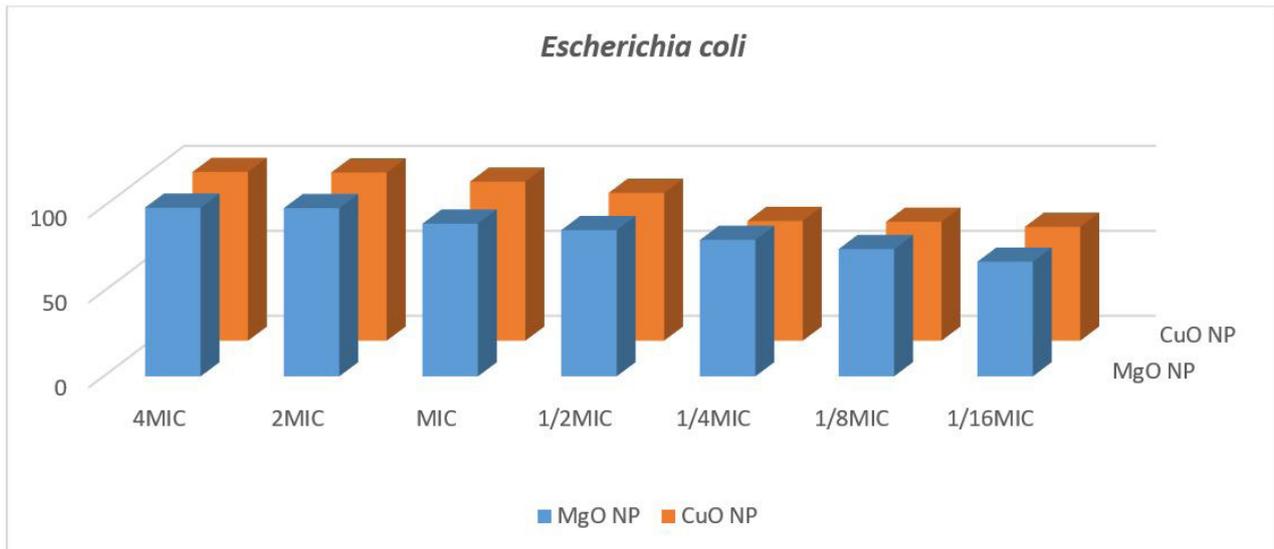


Figure 3: Percentage inhibition of *Escherichia coli* biofilm formation in the presence of different concentrations of nanoparticles

As shown in Figure 4, the average percentage of *Listeria monocytogenes* biofilm formation is suppressed when magnesium and copper oxide nanoparticles are present in different amounts.

Copper oxide nanoparticles were shown to be more effective at inhibiting *Listeria monocytogenes* biofilm formation. At the 1/4, 1/8, and 1/16 MICs, the inhibitory effects of the two nanoparticles differed significantly ($P < 0.05$), with the difference reaching approximately 23% at the 1/8 MIC.

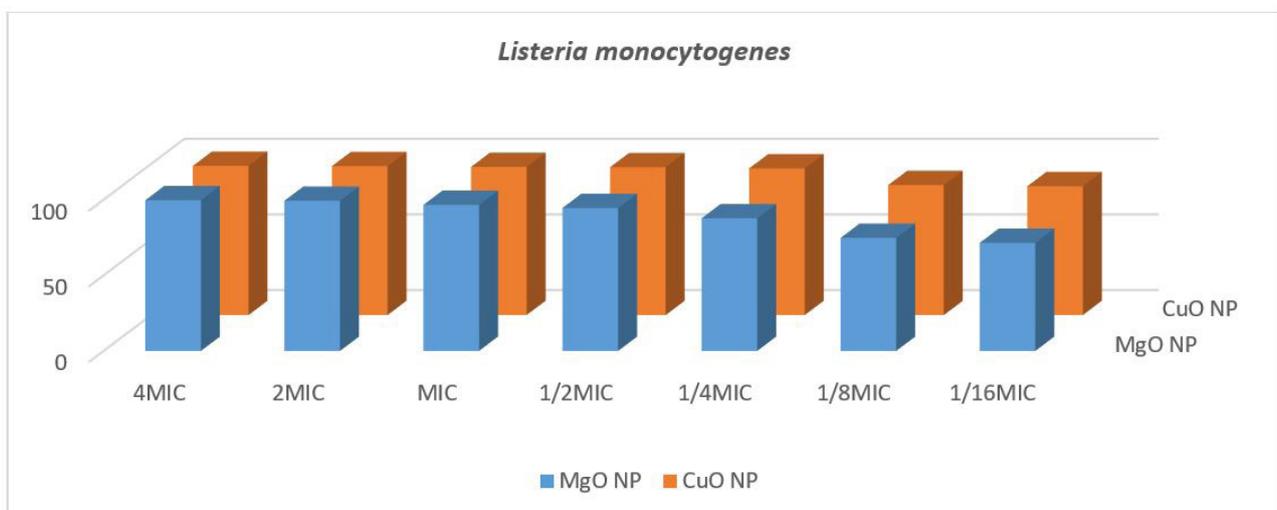


Figure 4: Percentage inhibition of *Listeria monocytogenes* biofilm formation in the presence of different concentrations of nanoparticles

As shown in Figure 5, the average percentage of *Staphylococcus aureus* biofilm formation is suppressed when magnesium and copper oxide nanoparticles are present in different amounts. By reducing the concentration of copper oxide, its ability to inhibit the formation of biofilm has decreased, so that in dilutions of 1.16, 1.8, and 1.4, the lowest inhibitory concentration was significant compared to dilutions of 4, 2, and 1 ($P < 0.05$). A similar trend has been observed in the case of magnesium oxide nanoparticle, so that the inhibition ability of this nanoparticle has decreased significantly ($P < 0.05$) in dilutions of 1.16 and 1.8, the lowest inhibitory concentration compared to other dilutions.

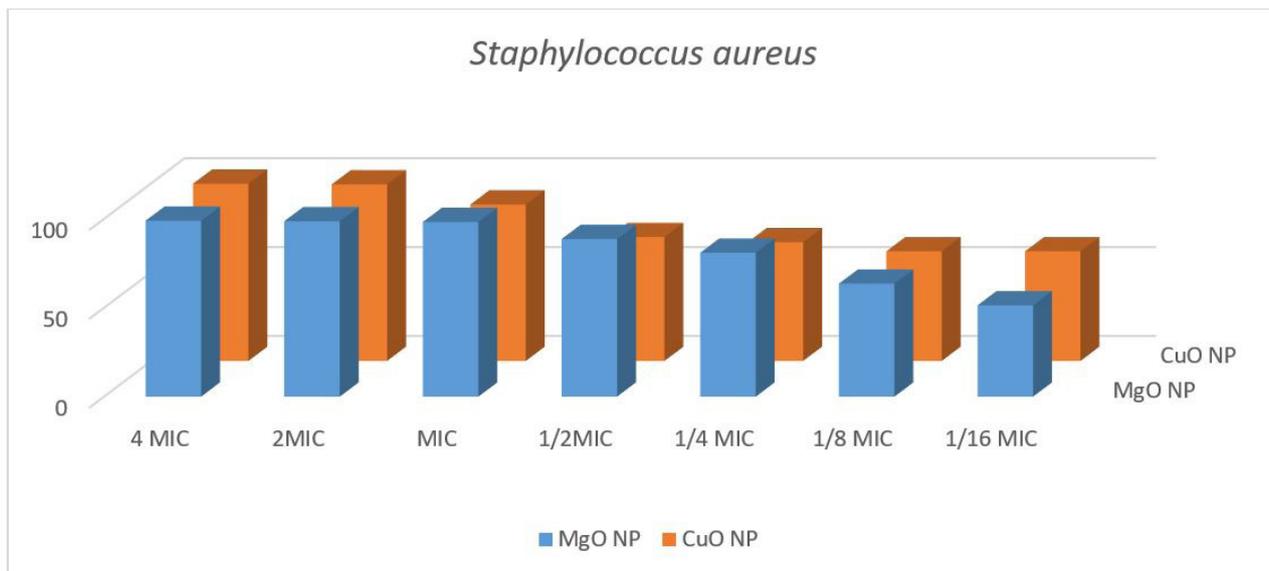


Figure 5: Percentage inhibition of *Staphylococcus aureus* biofilm formation in the presence of different concentrations of nanoparticles

As shown in Figure 5, the average percentage of *Pseudomonas aeruginosa* biofilm formation is suppressed when magnesium and copper oxide nanoparticles are present in different amounts. Following the decrease in the concentration of nanoparticles, their ability to inhibit biofilm formation has decreased, so that the inhibitory ability of magnesium oxide nanoparticles in dilutions of 1.16 and 1.8, the lowest inhibitory concentration, compared to dilutions of 4, 2, 1 and 1.2, has a significant decrease ($P < 0.05$) has found. Copper oxide also had the lowest inhibitory concentration and the least ability to inhibit biofilm formation in 1.16 dilution, which difference was significant compared to other dilutions ($P < 0.05$).

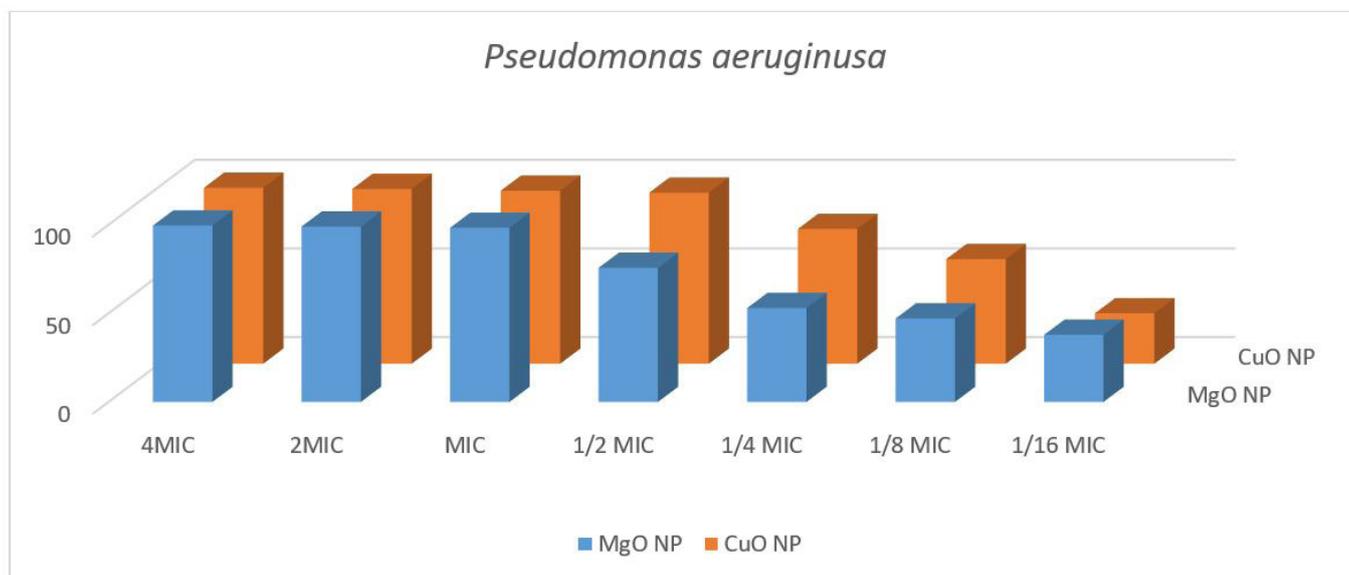


Figure 6: Percentage inhibition of *Pseudomonas aeruginosa* biofilm formation in the presence of different concentrations of nanoparticle

Discussion

This study demonstrated the antibacterial effects of magnesium oxide and copper oxide nanoparticles against *L. monocytogenes*, *E. coli* O157: H7, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Magnesium oxide nanoparticles had MICs of 2, 2, 2 and 4 mg/ml, while copper oxide nanoparticles had MICs of 1, 0.16, 0.5 and 0.25 mg/ml against *E. coli*, *L. monocytogenes*, *Staph. aureus* and *Paeruginosa* respectively.

The exact antibacterial mechanism of MgO nanoparticles is still unknown. A number of mechanisms, such as the formation of reactive oxygen species (ROS), the interaction of nanoparticles with bacteria, subsequently damaging the bacterial cell, and an alkaline effect have been proposed to explain the antibacterial mechanism of MgO nanoparticles [23]. Many studies have indicated that the antibacterial mechanism of MgO nanoparticles is due to the formation of ROS such as superoxide anion (O_2^-). It has been reported that the increase of the surface area of MgO particles leads to an increase of the O_2^- concentration in solution and thus results in a more effective destruction of the cell wall of the bacteria. However, when the particle size of MgO is below 15 nm, the aggregation effect becomes very significant due to the very high surface energy of the particles. The large size of aggregated MgO inhibits the interaction with bacteria and particles so that the bactericidal efficiency becomes lower [18]. Further, copper oxide nanoparticles have also been utilized to detect various chemicals, like hydrogen peroxide (H_2O_2), ammonia, glucose and lactic acid, etc. Due to its wide qualities, magnesium oxide has been investigated from a nutritional and health standpoint. Magnesium is an important mineral for human health [39].

Gokulakrishna et al. studied the antibacterial effect of five different metal nanoparticles, including magnesium nanoparticles, on pathogenic bacteria and discovered that *Streptococcus pneumoniae* was the most susceptible to magnesium oxide nanoparticles and *Klebsiella* was the least susceptible [16]. Numerous studies have shown that the antibacterial activity of magnesium oxide nanoparticles is linked to the production of reactive oxygen species such as superoxide anion. As the number of nanoparticles increases, the concentration of superoxide anion in the solution rises, causing more damage to the bacterial cell wall [41].

In a scientific study, Krishnamoorthy et al. discovered the mechanism of magnesium oxide nanoparticles' antibacterial action against the gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa*, as well as the gram-positive bacterium *Staphylococcus aureus*. For *E. coli*, the MIC was 0.5 mg/ml, and for *S. aureus* and *P. aeruginosa*, it was 1.0 mg/ml. They discovered that the susceptibility of bacteria to nanoparticles was linked not only to cell wall structure, but also to lipid peroxidation and the formation of reactive oxygen species [23].

Ingudam et al. investigated the antibacterial activity of magnesium oxide nanoparticles with an average size of 20 nm on a variety of foodborne pathogens such as *E. coli*, *Salmonella*, and *Campylobacter*. The microplate dilution method and resazurin reagent were used to determine MIC values in various nanoparticle concentrations with a bacterial inoculation level of 10^4 CFU/ml. The MICs for *E. coli*, *Salmonella*, and *Campylobacter* were 1, 2, and 0.5 mg/ml, respectively. Further research revealed that the difference between the negative charge of the bacterial cell and the positive charge of the nanoparticle acts as an electrostatic adsorbent, binding the nanoparticle to the cell surface [19]. In a study by Shekarforoush et al., they found that magnesium oxide nanoparticles and polylysine peptide had a good antimicrobial effect on *E. coli* O157: H7 and *L. monocytogenes*, inhibiting their growth at a concentration of 4.1 mg/ml. Using magnesium oxide nanoparticles as a potential antibacterial agent, either alone or in combination with other antimicrobial groups, could improve food safety, according to research findings [38].

Copper nanoparticles, both spherical and crystalline in shape, are used in a wide range of industries. The shape and size of nanoparticles influence their antimicrobial activity, with smaller particles having stronger and more antimicrobial activity than larger particles [35]. Yoon et al. investigated the antibacterial effects of copper oxide nanoparticles and silver oxide nanoparticles on *E. coli* and *Bacillus subtilis* in 2007, finding that copper oxide nanoparticles were more effective than silver nanoparticles. Copper and silver oxide nanoparticles can be coated on a variety of surfaces, including carbon or polyurethane foam, to combat germs [35].

Copper oxide nanoparticles have significant antibacterial activity against both gram-positive and gram-negative bacteria, according to Azam et al. They discovered that the size of these nanoparticles had an impact on their antibacterial properties [5]. Raffi et al. investigated the antibacterial efficacy of copper oxide nanoparticles against gram-negative *E. coli* in liquid and solid growth conditions. They believe that the antibacterial activities of copper oxide nanoparticles are primarily due to bacteria's surface tension (adhesion) [35]. If the MIC varies between studies, it could be due to changes in nanoparticle synthesis methods, nanoparticle size, and examined bacterial strains. Lipid peroxidation and the production of reactive oxygen species (ROS) may also influence the susceptibility of bacteria to nanoparticles [46]. According to a study by Abbasvali et al. on the inhibitory effect of zinc oxide nanoparticles on the biofilm formation of various foodborne pathogenic bacteria, zinc oxide nanoparticles have an antibacterial effect on gram-positive and gram-negative foodborne pathogenic bacteria. The minimum nanoparticle concentrations that could inhibit the growth of each bacterium were found to be 1, 0.5, 1 and 2 mg/ml for *E. coli*, *S. aureus*, *Salmonella typhimurium*, and *Bacillus cereus*, respectively [1].

Malakoutian et al. discovered that gram-positive *S. aureus* was more susceptible to three nanoparticles than gram-negative bacteria such as *E. coli* and *P. aeruginosa*, and could be killed at lower nanoparticle concentrations [27]. Because the outer membrane of gram-negative bacteria is mostly made of lipopolysaccharide (LPS), a nanoparticle resistant barrier, *E. coli* may be less sensitive to nanoparticles than other bacteria.

Petrus et al. concluded in their study that the presence of salts in the nutritional medium could enhance the effect of nanoparticle aggregation in suspension. Because nanoparticle size influences antimicrobial properties, nanoparticle aggregation properties may alter bactericidal efficacy and, as a result, MIC/MBC values [33].

According to the findings, coating key surfaces with CuO NPs and MgO NPs reduced bacteria population and biofilm formation. Biofilm formation has long been thought to be a critical mechanism that is clearly detrimental to the hygiene industry (Marques et al. 2007). A number of factors influence bacterial adhesion to different surfaces, including hydrophobicity, cell-surface charge, electron acceptor and donor characteristics [6].

According to Cerca et al., a high degree of hydrophobicity is required for biofilm formation [9]. Lee and Yii discovered that *P. aeruginosa* and *L. monocytogenes* have high levels of hydrophobicity, which could explain their tendency to stick to surfaces [24].

Furthermore, the results of this research show that CuO NPs and MgO NPs have inhibitory effects on a wide range of bacterial species. There were differences in sensitivity to NPs between the two bacterial species. This confirms previous findings that the antibacterial action of NP is influenced by microbial species. For example, according to Kim et al., different microbes have varying antimicrobial susceptibilities to nanoparticle [22].

After studying the antibacterial activities of silver nanoparticles and copper nanoparticles, researchers found that the sensitivity of microbes to nanoparticles depends on the species of microbe [36]. The findings of this study are in line with those of previous studies.

The surface area available to interact with nanoparticles determines their antibacterial activity on bacteria. When the accessible surface area for interactions of nanoparticles (NPs) is increased, their antibacterial efficacy and cytotoxicity are increased [35]. Furthermore, because smaller nanoparticles have a larger contact area, they can be more active than larger particles [29].

Because CuO NPs and MgO NPs inhibit bacterial adhesion, the significant reduction in biofilm formation on surfaces coated with NPs could be attributed to their inhibitory effect. Our findings confirm the antibiofilm behavior of CuNP-coated surfaces and, as such, are consistent with the findings of an earlier study by Eshed et al [14]. When considering the rise in industrial contamination, particularly in the food industry, the importance of eliminating microbial contamination on surfaces becomes clear.

Conclusion

Magnesium oxide nanoparticles had MICs of 2, 2, 2 and 4 mg/ml, while copper oxide nanoparticles had MICs of 1, 0.16, 0.5 and 0.25 mg/ml against *E. coli*, *L. monocytogenes*, *Staph. aureus* and *Paeruginosa* respectively. An increasing number of bacteria are resistant to antimicrobial treatments, making this task even more important. In this study, CuO NPs and MgO NPs were tested for antibacterial activity against *L. monocytogenes*, *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. CuO nanoparticles and MgO nanoparticles have the potential to reduce the negative effects of microorganisms.

Conflicts of Interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

References

1. Abbasvali M, Ebrahimi Kahrizsangi A, and Shahriari F (2017) Evaluation of the Inhibitory Effects of Zinc Oxide Nanoparticles on Biofilm Formation of Some Foodborne Bacterial Pathogens. *Iranian Journal of Medical Microbiology*, 11: 115-124.
2. Adetunji V, Adedeji AO, and Kwaga J (2014) Assessment of the contamination potentials of some foodborne bacteria in biofilms for food products. *Asian Pacific Journal Tropical Medicine*, 7: 232-237.
3. Agarwala M, Choudhury B, Yadav R.N.S (2014) Comparative Study of Anti Biofilm Activity of Copper Oxide and Iron Oxide Nanoparticles Against Multidrug Resistant Biofilm Forming Uropathogens. *Indian Journal of Microbial*, 54: 365–368.
4. Anan S, Singh D, Avadhanula M, and Marka S (2014) Development and control of bacterial biofilms on dairy processing membranes. *Comp Rev Food Sci Food Safety*, 13:18-33.
5. Azam A (2012) Size-dependent antimicrobial properties of CuO nanoparticles against gram-positive and negative bacterial strains. *International Journal of Nano medicine*, 27:27-35.
6. Briandet R, Meylheuc T, Maher C, Bellon Fontaine MN (1999) *Listeria monocytogenes* Scott A: cell surface charge, hydrophobicity, and electron donor and acceptor characteristics under different environmental growth conditions. *Appl Environ Microbiol*, 65:5328–5333.
7. Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbial*, 94: 223-253.
8. Camargo A, Woodward J J, Call D R, and Nero L A (2017) *Listeria monocytogenes* in food-processing facilities, food contamination, and human listeriosis, The Brazilian Scenario. *Foodborne Pathog. Dis*, 14: 623–636. doi: 10.1089/fpd.2016.2274.
9. Cerca N, Pier GB, Vilanova M, Oliveira R, Azeredo J (2005) Quantitative analysis of adhesion and biofilm formation on hydrophilic and hydrophobic surfaces of clinical isolates of *Staphylococcus epidermidis*. *Res Microbial*, 156:506–514.
10. Colagiorgi A, Bruini I, Di Ciccio P A, Zanardi E, Ghidini S, and Ianieri A (2017) *Listeria monocytogenes* biofilms in the wonderland of food industry. *Pathogens*, 6: E41. doi: 10.3390/pathogens6030041.
11. Costerton JW, Irvin RT, Cheng KJ (1981) The bacterial glycocalyx in nature and disease. *Ann Review Microbial*, 35:299-324.
12. Dunsmore DG, Twomey A, Whittlestone WG, Morgan HW (1981) Design and performance of systems for cleaning product-contact surfaces of food equipment, a review. *Journal of Food Protect*, 44: 220-240.
13. Emamifar A, Kadivar M, Shahedi M, SoleimaniZad S (2011) Effect of nanocomposite packaging containing Ag and ZnO on inactivation of *Lactobacillus plantarum* in orange juice. *Food Control*, 22:408-413.
14. Eshed M, Lellouche J, Matalon S, Gedanken A, Banin E (2012) Sonochemical coatings of ZnO and CuO nanoparticles inhibit *Streptococcus mutans* biofilm formation on teeth model. *Langmuir*, 2028:12288–12295.
15. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, and Kjelleberg S (2016) Biofilms: an emergent form of bacterial life. *Nat Microbial*, 14:563. doi:10.1038/nrmicro.

16. Gokulakrishnan R, Ravikumar S, Raj JA (2012) In vitro antibacterial potential of metal oxide nanoparticles against antibiotic resistant bacterial pathogens. *Asian Pac Journal Trop Dis*, 2: 411-413.
17. Herigstad B, Hamilton M, Heersink J (2001) How to optimize the drop plate method for enumerating bacteria. *Journal of Microbial Methods*, 44:121–9.
18. Huang L, Li D Q, Lin Y J, Wei M, Evans D G, Duan X (2005) Controllable preparation of Nano MgO and investigation of its bactericidal properties. *Journal of Inorganic Biochemistry*, 99: 986- 993.
19. Ingudam SH, Irwin P, Reed S, He Y (2012) Antibacterial activity of magnesium oxide nanoparticles on *Escherichia coli* O157:H7, *Salmonella*, and *Campylobacter*. *Journal of Nanomed Nanotechol*, 3:78.
20. Jin T, He Y (2011) Antibacterial activities of magnesium oxide (MgO) nanoparticles against foodborne pathogens. *Journal of Nanopart Research*, 13:6877–6885.
21. Khelissa S O, Abdallah M, Jama C, Faille C, and Chihib N E (2017) Bacterial contamination and biofilm formation on abiotic surfaces and strategies to overcome their persistence. *Journal of Mate. Environ. Sci*, 8:3326-3346.
22. Kim JS, Kuk E, Yu KN, Kim J H, Park S J, Lee H J, et al (2017) antimicrobial effects of silver nanoparticles. *Journal of Nano medicine*, 3: 95–101.
23. Krishnamoorthy K, Manivannan G, Kim SJ, Jeyasubramanian K, Premanathan M (2012) Antibacterial activity of MgO nanoparticles based on lipid peroxidation by oxygen vacancy. *Journal of Nano part Research*, 14:1063-1066.
24. Lee KK, Yii KC (1996) A comparison of three methods for assaying hydrophobicity of pathogenic vibrios. *Letters Appl Microbial*, 23:343–346.
25. Lei H, Dianqing L, Yanjun L, Evans DG, Xue D (2005) Influence of Nano-MgO particle size on bactericidal action against *Bacillus subtilis* var. *Niger*. *Chinese Science Bulletin*, 50(6):514-519.
26. Mahdavi M, Jalali M, Kasra K R (2008) The Assessment of Biofilm Formation in Iranian Meat Processing Environments. *Research Journal Microbial*, 3(3):181–186.
27. Malakootian M, Toolabi A (2010) Determining and comparing the effect of nanoparticle CuO, TiO₂ and ZnO in removing gram positive and gram-negative bacteria from waste water. *Journal of Tolooe Behdasht*, 9: 1-11.
28. Marques SC, Rezende J, Alves L, Silva BC, Alves E, Abreu, LR et al (2007) Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. *Brazilian Journal Microbial*, 38:538–543.
29. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, et al (2007) The bactericidal effect of silver nanoparticles. *Nanotechnology*, 16(10):2346–2353.
30. Nikolic M, Vasic S, Durdevic J, Stefanovic O, Comic L (2014) Antibacterial and anti-biofilm activity of ginger (*Zingiber Officinale* (Roscoe)) ethanoic extract. *Kragujevac J Sic*, 36:129-136.
31. Noori A J, Kareem F A (2010) The effect of magnesium oxide nanoparticles on the antibacterial and ant biofilm properties of glass-ionomer cement. *Heliyon*, 5: e02568.

32. Parsek M R, Fuqua C (2004) Emerging themes and challenges in studies of surface-associated microbial life. *Journal of Bacteriology*, 186: 4427-4440.
33. Petrus EM, Tinakumari S, Chai LC, et al (2011) A study on the minimum inhibitory concentration and minimum bactericidal concentration of Nano colloidal Silver on food-borne pathogens. *International Food Research*, 18: 55-66.
34. Pintucci JP, Corno S, Garotta M (2010) Biofilms and infections of the upper respiratory tract. *Eur. Rev. Med. Pharmacol. Sci*, 14: 683-690.
35. Raffi M, Mehrwan S, Bhatti T, et al (2010) Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli*. *Journal of Annals of Microbiology*, 60: 75-80.
36. Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S (2010) Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomater*, 4:707-716.
37. Sarker SD, Nahar L, Kumarasamy Y. (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, 42 (4):321-324. doi: 10.1016/j.ymeth.2007.01.006.
38. Shekarforoush S, Samadi M, Gheisari H R (2016) Antimicrobial effects of magnesium oxide nanoparticles and ϵ -Poly-L-lysine against *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Iranian Journal of Medical Microbiology*, 10: 33-41.
39. Shi L, Xing L, Hou B, et al (2016) Inorganic Nano metal oxides used anti microorganism agents for pathogen control. *Journal of Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, 361-368.
40. Stepanovic S, Vukovic D, Hola V, Bonaventura GD, Djukic S (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *Apmis*, 115:891-899.
41. Tang ZH, Lv BF (2014) MgO nanoparticles as antibacterial agent: preparation and activity. *Brazilian Journal of Chemical Engineering*, 31:591-601.
42. Tendolkar PM, Baghdayan AS, Gilmore MS, Shankar N (2004) Enterococcal surface protein, Esp, enhances biofilm formation by *Enterococcus faecalis*. *Infect Immun*, 72:6032-6039.
43. Tong M, Ding J, Shen Y, and Zhu P (2010) Influence of biofilm on the transport of fullerene (C60) nanoparticles in porous media. *Water Res*, 44: 1094-1103.
44. Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine*, 12: 1227-124.
45. Yoon K, Hoon Byeon J, Park J (2007) Susceptibility constants of *Escherichia coli* and *Bacillus subtilis* to silver and copper nanoparticles. *Journal of Science of the Total Environment*, 373:572-575.
46. Yousef J M, Danial EN (2012) In vitro antibacterial activity and minimum inhibitory concentration of zinc oxide and nano-particle zinc oxide against pathogenic strains. *Journal of Health Science*, 2:38-42.