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Testing of Antimicrobial Effectiveness of Silver Nitrate Nanoparticles

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Abstract **Original Research Article**

The spread of epidemics in recent years has underscored the essential role of disinfection. Current methods have begun to be inadequate for the disinfection of new epidemics, viruses and resistant microorganisms. Silver nanoparticles (Ag NPs) are recognized as the most preferred and ideal product within the realm of nanotechnology among all metallic nanoparticles (noble metals). Silver nanoparticles are used in many sectors, including medical, food, and cleaning products, due to their high conductivity, catalytic properties, chemical stability, and anti-inflammatory and antiviral activity. The risk of infection is very high in crowded areas such as hospitals, homes, schools and work environments. No matter how much attention is paid to the cleaning of communal living spaces, it is insufficient for today's epidemic diseases. Due to the prevalence of cross-contamination, the importance of disinfection of personal hygiene, work and shelter environments comes to the fore once again. Resistant microorganisms continue to show their activity on surfaces shortly after disinfection processes. For this reason, the use of silver nitrate nanoparticles, which have higher microorganism resistance on surfaces, can prevent these problems. In this study, the silver nitrate nanoparticle-added solution showed the expected success against microorganisms.

Keywords: Silver nitrate, Nanoparticle, Disinfection, Epidemic, SEM.

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1. INTRODUCTION

Nanoscience and nanotechnology are thought to be the brightest areas in science and technology. For this reason, interest in nanoscience and nanotechnology has been increasing recently. Recently, every country in the world has been making significant investments in this field (Salerno et al., 2008). Nanotechnology science; It is a field that guides science by combining information from the fields of physics, chemistry, biology, medicine, informatics and engineering. Thanks to nanotechnology, a technological field is being formed with major innovations that can be applied to real life. With the use of nanotechnology tools that can examine the functions, properties and reactive mechanisms of living or non-living materials smaller than 100 nm with nanotechnology science, new nano biomaterials and nano devices can be manufactured and controlled. Nanoparticles have large surface areas and good conductivity (Sreekanth et al., 2016). Nanoparticles are widely used in many areas such as biomedicine, pharmacology, bioremediation, food, cosmetics, agricultural applications (El-Batal et al., 2018). They are synthesized by various methods such as chemical, physical and biological methods. However, biological methods are preferred more due to their advantages such as being environmentally friendly and easy. Bacteria, algae, fungi and plant sources are used in synthesis with biological methods (Swamy et al., 2015).

In recent years, the resistance of pathogenic bacteria to antimicrobial agents has become an important health problem. Since some antimicrobial agents are extremely toxic and irritating, there is great interest in the development of new types of reliable and cost-effective antimicrobial materials. For these reasons, silver nanoparticles are known to have inhibitory and bactericidal effects and their use has been focused on. The antibacterial properties of silver ions were discovered in the 19th century. In the 1920s, the US Food and Drug Administration (FDA) recognized colloidal silver as an effective treatment for wounds. However, with the discover of penicillin in the 1940s, antibiotics became the preferred method for treating bacterial infections, leading to a decline in the use of silver ions (Chopra, 2007).

Silver nanoparticles adhere to the bacterial cell membrane and also pass into the bacteria. When silver nanoparticles enter the bacterial cell, they interact with compounds such as sulfur-containing proteins and phosphorus-containing DNA. In such a case, although they create a low molecular weight region to protect the bacterial DNA, silver ions affect the respiratory chain and cause the bacteria to die (Rai et al., 2009). In hand disinfectant applications, products that pass the first day tests in particular may not pass the microorganism and virus tests. In such a case, formulators generally prefer to use high % v/v alcohol and the second active ingredient. As a result of this action taken by the formulator, the product causes high damage to the hand while passing the microorganism tests, causing irritation and failing the test and not passing the registration stage. Although high percentage (82.7% v/v) ethyl alcohol is used in the Mitreapel liquid disinfectant product, high results were obtained in terms of irritation with the correct amount of moisturizing glycerin. In the Mitreapel spray disinfectant product, although the amount of ethyl alcohol (73.43% v/v) was reduced, a second supporting active substance, hydrogen peroxide (0.05% v/v), was used to pass microorganism and virus tests (Güzel et al., 2022). The use of silver nitrate nanoparticles with high antibiotic bacterial resistance has gained importance in the health field nowadays. The aim of this study is to evaluate the antimicrobial effect of silver nitrate nanoparticles using a synthesis method. Sprays to be obtained with silver nitrate nanoparticles have the potential to be used in disinfection and hygiene applications. According to their antimicrobial effect, silver nitrate nanoparticles tend to damage the cell membranes and genetic materials of microorganisms. Therefore, silver nitrate nanoparticle spray is effective in a wide microbial spectrum. Silver nanoparticles (Ag NPs) are regarded as one of the most promising and extensively utilized nanomaterials, particularly among metallic nanoparticles, especially those of noble metals. They are distinguished by their outstanding electrical conductivity, catalytic effectiveness, chemical resilience, and, most significantly, their potential to exhibit anti-inflammatory and antiviral properties (Ahmed et al., 2016). Silver nitrate nanoparticles can adhere to surfaces and maintain their antimicrobial effects for a long time. It can provide longer-term protection after application. It is difficult for microorganisms to develop resistance to silver nitrate nanoparticles. This increases the long-term effectiveness of silver nitrate nanoparticle spray. It does not irritate the skin and can be a more convenient option for users. Silver nitrate nanoparticle spray formulations are environmentally friendly and biodegradable. It is odorless and colorless, which ensures that there are no unpleasant odors or color changes on the surfaces it is used on. The spray formula can be easily applied to surfaces and can be used in different areas that need to be disinfected. It has a wide range of uses. Silver nitrate nanoparticle sprays can be used appropriately for healthcare institutions, food industry, home disinfection and many other areas. While the exact antibacterial mechanism of Ag NPs remains unclear, various aspects of their antimicrobial properties have been identified. Silver ions can interact with the cellular structures of numerous bacteria (Takahashi, 2015). Silver nitrate nanoparticles are nanometer-scale particles of silver nitrate salt. Nanoparticles are materials with dimensions between 1 and 100 nanometers. Silver nitrate nanoparticles generally have antimicrobial properties and can be used in various applications, especially in antibacterial coatings and medical fields. The small size of these particles increases the surface area, which makes them particularly effective. Silver nitrate nanoparticles are very small-sized particles obtained by molecular decomposition of silver nitrate. They usually have diameters ranging from 1 to 100 nanometers. These small sizes increase the large surface area and reactivity of silver nitrate, resulting in various properties. These nanoparticles can be used in antibacterial, antiviral and antifungal applications due to their antimicrobial properties (Hutchison, 2008). For example, silver nitrate nanoparticles can be used to inhibit the growth of microorganisms in surface coatings or medical products. This could be a potentially effective strategy to reduce the risk of infection in various medical devices and materials. In addition, silver nitrate nanoparticles can have catalytic properties, which can increase the speed of chemical reactions and allow them to be used in certain applications. Such properties lead to a wide range of applications in the fields of nanotechnology and materials science. Silver nitrate nanoparticles can also be used in areas such as photocatalysis, sensor technology and optoelectronic applications due to their high surface area and special properties. In particular, their photocatalytic properties refer to their ability to cause chemical reactions when exposed to light. This property creates potential uses in various applications such as water treatment, air purification or energy production. Silver nitrate nanoparticles can also play a role in biomedical applications (Akköz et al., 2019). They can be found among nanotechnology-based solutions that can be used especially in the fields of imaging and treatment. The biocompatibility of nanoparticles and their ability to interact with biological systems have the potential to provide innovative solutions in the field of medicine. Silver nitrate nanoparticles may also have various applications in electronics. In particular, the conductive properties of silver nanoparticles can be evaluated in flexible electronics, nanotechnology and microelectronics applications (Yavuz et al., 2021; Uslan, 2023). This allows them to be used in areas such as flexible electronic devices, sensors or display technologies. The high surface area of nanoparticles provides a suitable environment for electrocatalytic properties. This can offer various technological solutions that can be used in applications such as biosensors, fuel cells and electrical storage devices. Silver nitrate nanoparticles can be considered as a potential material for miniaturization and improved performance of electronic components. Silver nitrate nanoparticles may have antibacterial and antiviral properties. These properties can be used in surface coatings, medical devices and dressings to prevent or stop the growth of microorganisms. Nanoparticles can be used as contrast agents in biomedical imaging techniques (Abdulgani, 2023). They can provide improved contrast, especially for magnetic resonance imaging (MRI) or optical imaging techniques. Silver nitrate nanoparticles can be used as drug carriers. They can direct drugs to a targeted area and thus increase the effectiveness of the treatment. Photodynamic therapy is a method of killing cells using light-sensitive substances. Silver nitrate nanoparticles can be used as potential carriers for photodynamic therapy. Nanoparticles can be used in biolabeling applications to track cells or biological molecules. This may be important for understanding cell behavior and developing therapeutic strategies. The use of silver nitrate nanoparticles in these areas continues to be an ongoing research topic to innovate biomedical research and treatment strategies (Araz, 2017). Ag NPs are recognized for their broad-spectrum antibacterial effects against both Gram-positive and Gram-negative bacteria, including strains that are resistant to antibiotics. The antibacterial mechanism of Ag NPs mainly stems from the release of silver ions. When employing small Ag NPs (less than 10 nm), the release of silver ions is notably more effective than that from larger particles (Bapat et al., 2018). Silver ions interact with electron donor groups in nitrogen-containing organic compounds, resulting in the formation of holes in the bacterial membrane, which leads to the leakage of cellular contents and ultimately bacterial death (Damm et al., 2008). It is now established that silver-based compounds are detrimental to microorganisms. Consequently, various medical systems have been developed to deliver silver ions for antibacterial applications. A proven strategy to enhance their efficacy is by increasing the relative surface area through the reduction of silver particle size (i.e., NPs) (Kaur et al., 2016; Rajeshkumar, 2016). Silver nitrate nanoparticles compromise the peptidoglycan layer of the bacterial cell wall, while silver ions disrupt the cell membrane. These ions also bind to DNA bases, inducing DNA condensation, which inhibits bacterial cell division and interrupts binary fission. Furthermore, they disrupt protein synthesis by destabilizing ribosomes and contribute to the breakdown of the plasma membrane (Mitthra et al., 2019). Ag NPs exhibit antiseptic properties against Gram-negative bacteria and induce significant structural damage, creating deep cavities in their cell walls (Sondi et al., 2004). In addition, silver NPs produce free radicals upon encountering bacteria; these radicals compromise the integrity of the cell membrane, rendering it porous and ultimately leading to cell death (Danilczuk et al., 2006).

2. MATERIAL AND METHODS

In this study, silver nano nanomaterial solution was developed and its antimicrobial effect was observed. The work flow chart is given in Figure 1.

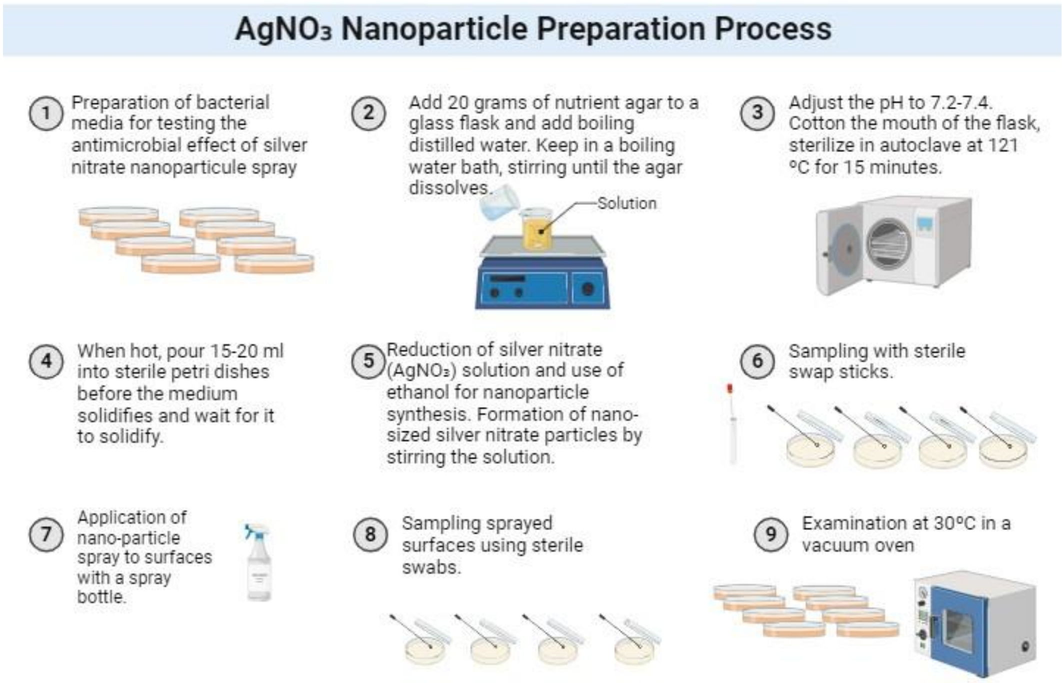


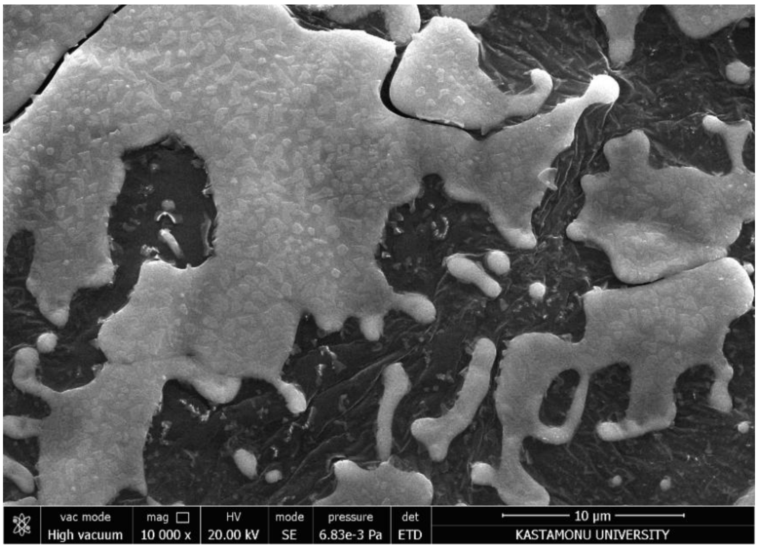
Figure 1. Preparation of Ag Nanoparticles and evaluation of their antimicrobial effect.

Silver nitrate solution with 0.1 normality was supplied by Kimyalab company. Bacterial media were prepared to test the antimicrobial effect of silver nitrate nanoparticle spray. 20 grams of nutrient agar and boiling sterile water were added to the glass flask. The agar was kept in a boiling water bath by stirring until it melted. Its pH was adjusted to 7.2–7.4. The mouth of the flask was cottoned and sterilized in an autoclave at 121 ºC for 15 minutes. While hot, before the medium solidified, 15–20 ml were poured into sterile petri dishes and waited for solidification. Ethanol was used as the reducing agent for reduction to silver nitrate ($AgNO_3$) solution and nanoparticle synthesis. Nano-sized silver nitrate particles were formed by stirring the solution. The resulting nanoparticle solution was stabilized by stirring at constant temperature for a while. The nanoparticle solution was transferred to the spray bottle. Samples were taken from Figure 6.A door handle, Figure 7.A table surface, Figure 8.A trash can and Figure

9.A sink surface with sterile swab sticks and inoculated into bacterial culture. In the second stage, nanoparticle spray was carefully applied to the same surfaces with a spray bottle to ensure an even distribution. After that, samples were taken from the surfaces Figure 6.B door handle, Figure 7.B table, Figure 8.B trash can and Figure 9.B sink surface with sterile swab sticks and inoculated into bacterial culture. It was examined in a vacuum oven at 30ºC degrees.

3. RESULTS

SEM and EDS data were obtained from the sample obtained from the produced spray. In these figures (Figure -2), $AgNO_3$ nanoparticles are clearly seen.



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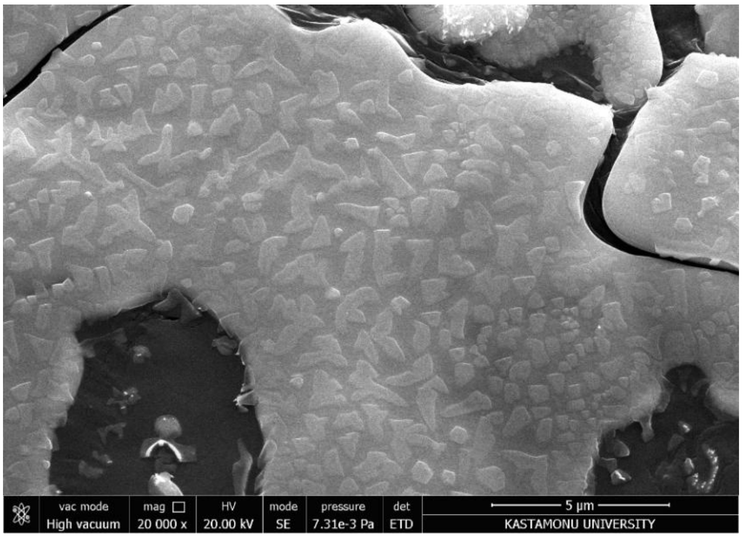

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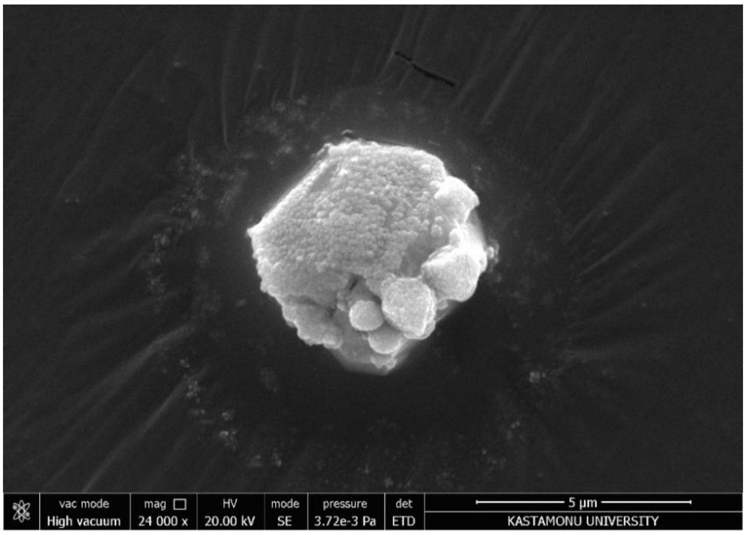
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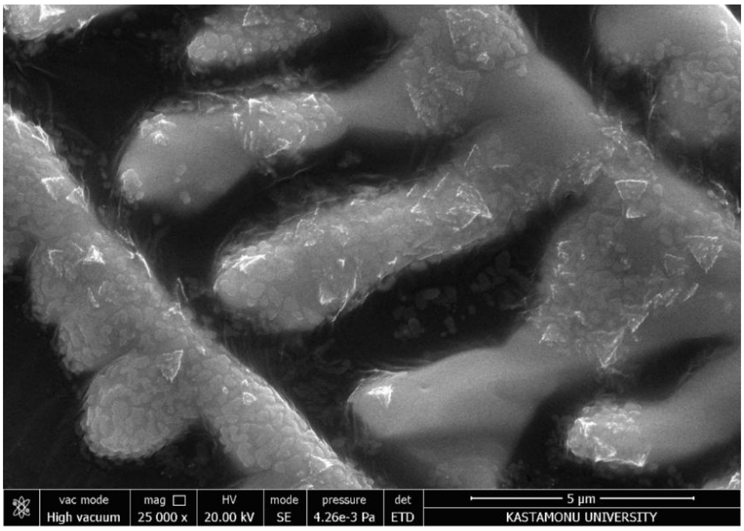
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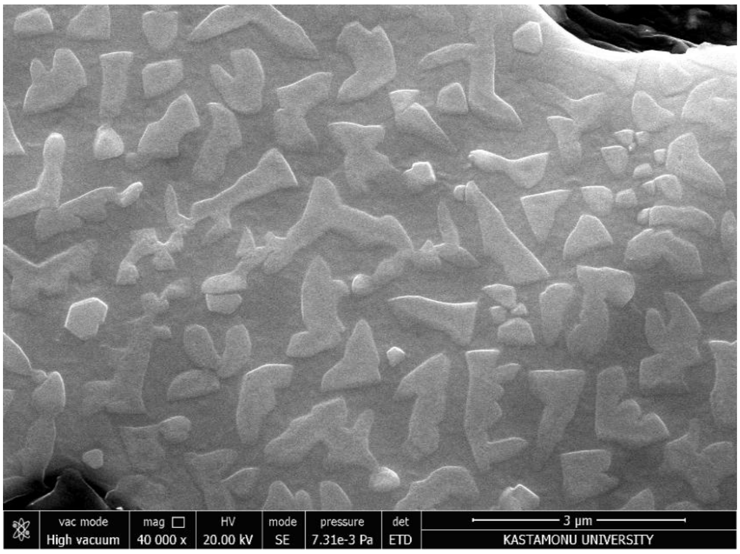
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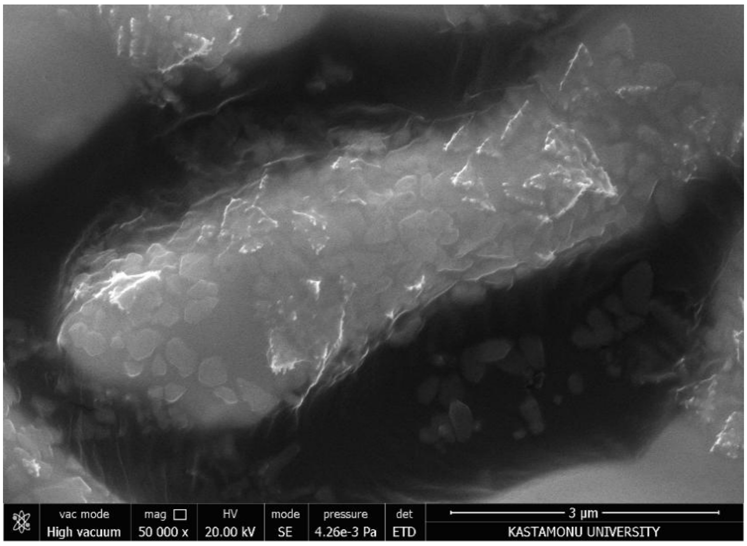


f)

Figure 2 $AgNO_3$ nanoparticles SEM images at different magnification types a) 10000x b) 20000x c) 24000x d) 25000x e) 40000x f) 50000x

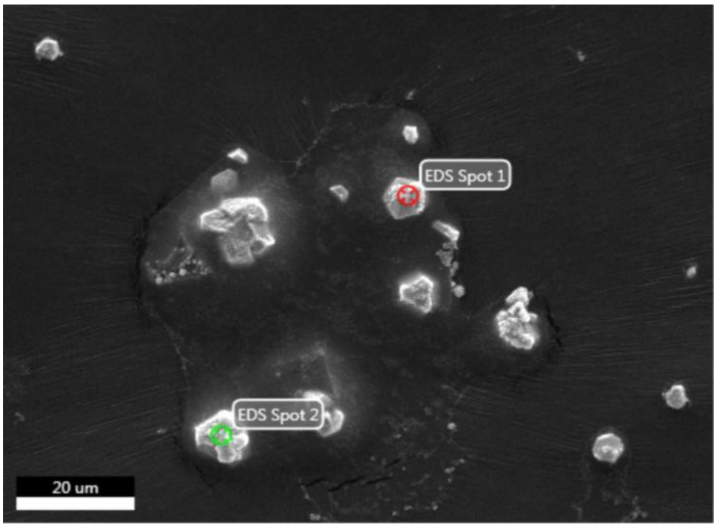
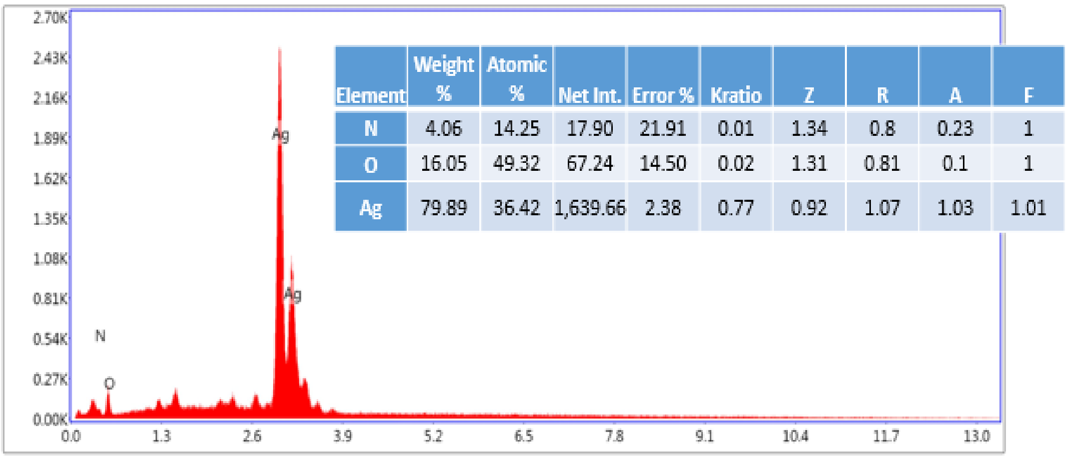
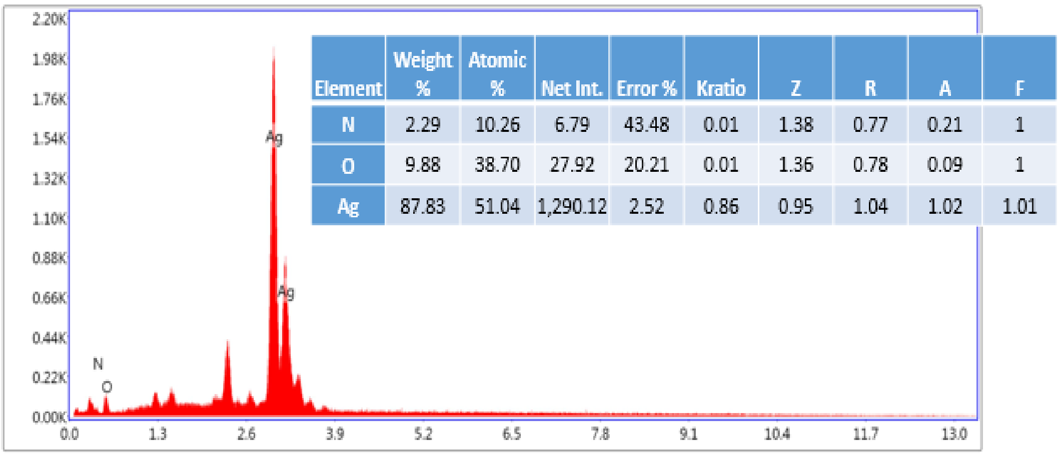


Figure 3 Representation of EDS spots from the example (EDS Spot 1 & EDS Spot 2)



Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
N	4.06	14.25	17.90	21.91	0.01	1.34	0.8	0.23	1
O	16.05	49.32	67.24	14.50	0.02	1.31	0.81	0.1	1
Ag	79.89	36.42	1,639.66	2.38	0.77	0.92	1.07	1.03	1.01

Figure 4 Spot 1 EDS data



Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A	F
N	2.29	10.26	6.79	43.48	0.01	1.38	0.77	0.21	1
O	9.88	38.70	27.92	20.21	0.01	1.36	0.78	0.09	1
Ag	87.83	51.04	1,290.12	2.52	0.86	0.95	1.04	1.02	1.01

Figure 5 Spot 2 EDS data

There are N, O, and Ag elements composition in EDS Spot 1. They are separated from each other for % weight; 4.06, 16.05 and 79.89, N, O and Ag, respectively. Similarly, they are differed from each other for % atomic composition; 14.25, 49.32 and 36.42 with same order like previous one. In the same order for % weight like Spot 1, N is 2.29, O is 9.88 and Ag is 87.83 for Spot 2. There is a change in the order of % atomic composition which are Ag is the first 51.04, O is the second now 38.70. N is still last one for atomic composition which is 10.26.

For the visual examination, to observe the effect of silver nitrate nanoparticle spray, the samples taken from the door handle (Figure 6), from the table surface (Figure 7), from the trash can (Figure 8), and from the sink surface (Figure 9) were viewed after 7 days. (The samples were taken from the Biomedical Engineering Laboratory of the Faculty of Engineering and Architecture of Kastamonu University.)

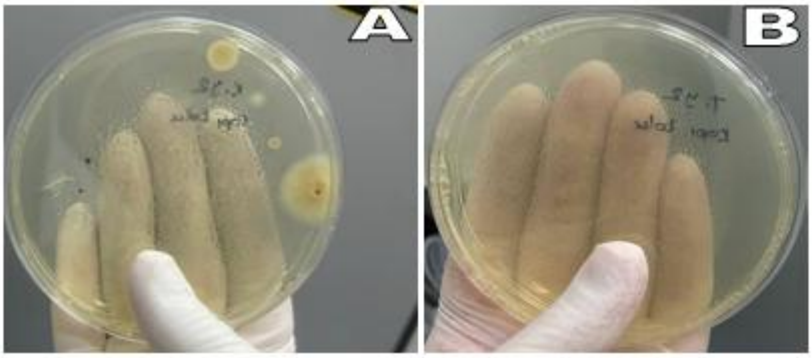


Figure 6.A. Bacteria proliferation in samples taken from door handles using swab.
B. Bacterial proliferation in samples taken from door handles using swabs following the application of a silver nitrate nanoparticle solution spray.

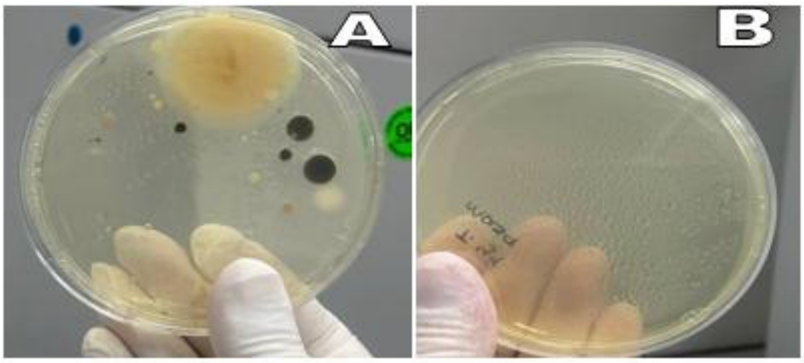


Figure 7.A. Bacterial formation in swab samples taken from the table surface.
B. Bacterial formation in swab samples taken from the table surface with a swab stick after applying silver nitrate nanoparticle solution spray.

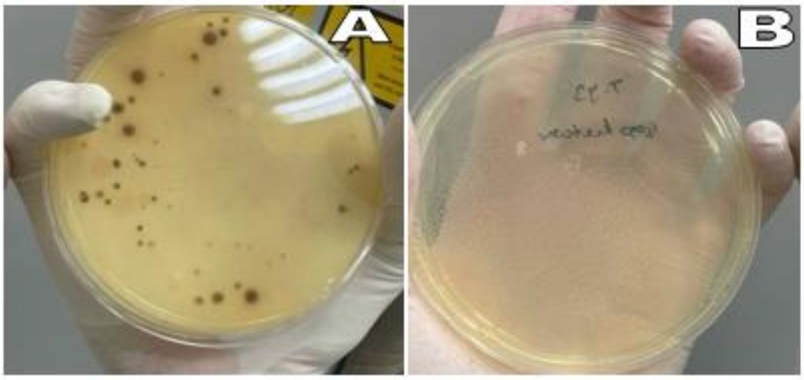


Figure 8.A. Bacterial formation in swab samples taken from the trash can.
B. Bacterial formation in swab samples taken from the trash can with a swab stick after applying silver nitrate nanoparticle solution spray.

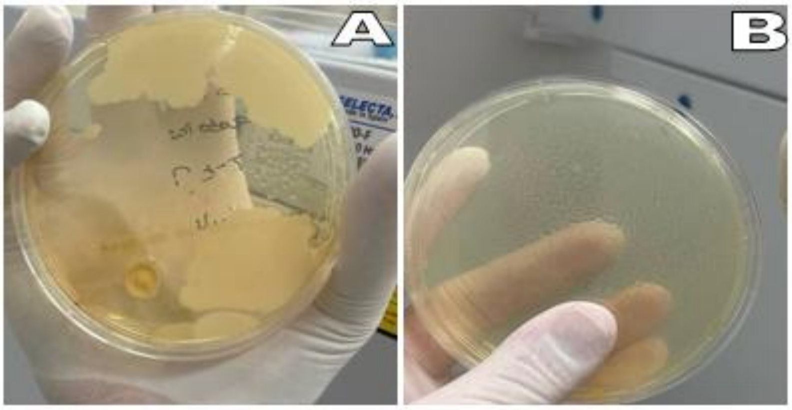


Figure 9.A. Bacterial growth detected in swab samples collected from the sink surface.
B. Bacterial formation in swab samples taken from the sink surface with a swab stick after applying silver nitrate nanoparticle solution spray.

The evaluation of these results was made by visual evaluation of swabs taken from various surfaces after 7 days. During this period, a rapid increase in the bacterial population was observed in petri dishes without solution, while the bacterial growth was extremely limited in petri dishes with solution.

4. DISCUSSION & CONCLUSION:

Silver nitrate nanoparticles have antimicrobial properties and can be used in medical fields. Especially in disinfection processes that have become very important today, the use of silver nitrate can provide long-term disinfection. Prepared bacterial media were examined in a vacuum oven for 7 days. Very limited bacterial growth was observed in the media containing samples taken from surfaces where silver nitrate nanoparticle spray was applied for 7 days. Considering that this solution is used in environments such as hospitals, it will provide great convenience in the disinfection process of devices that have a high risk of contamination and are not suitable for sterilization. Silver nitrate provides long-term disinfection on surfaces due to its surface adhesion and microbial resistance properties. This long-term disinfection will significantly reduce the cleaning burden, especially in hospitals. In addition to the areas used by patients in hospitals, it can also be used in areas where hospital staff eat, reducing the spread in this area where the risk of contamination of hospital staff is common. It can be a disinfection material that everyone can easily access due to its low cost. It can reduce the rate of disease spread by increasing its use in areas important for human health such as hospitals, schools, homes, businesses and the food sector. It can also be used as a hand disinfectant due to its biocompatibility. Since it has a wide range of uses, many areas of use can be provided with a single product. It shows that silver nitrate nanoparticle spray has a positive antimicrobial effect on certain microorganisms on the surfaces it was tested on. This indicates that the spray can be evaluated as a potential cleaning and microbial prevention method. These results support the idea that silver nitrate nanoparticles can potentially be used in microbial control and cleaning applications.

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CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

CREDIT AUTHOR STATEMENT

1st Author: Investigation, Conceptualization, Methodology. **2nd Author:** Investigation, Conceptualization, Methodology. **3rd Author:** Data curation, Writing- original draft. Writing- review and editing. **4th Author:** Visualization, Writing- review and editing. **5th Author:** Supervision, Validation, Writing- review and editing.

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