INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE
ISSN: 2067-533X Volume 17, Issue 2, 2026: xxx-xxx www.ijcs.ro
DOI: 10.36868/IJCS.2026.02.21

ANTIBACTERIAL COATINGS FOR THE PROTECTION OF RELIGIOUS ARTIFACTS

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Abstract
Nowadays, epidemics related to the excessive emission of viruses and bacteria are very common. Society is becoming increasingly susceptible to viral and bacterial infections, which are becoming more difficult to combat. The increased number of available, not fully tested vaccines and antibiotics produced in the laboratories of pharmaceutical companies does not contribute to solving this problem. 90% of superficially tested medicines are a temporary solution that does not address the problem in a generational or long-term sense. This statement is often controversial, but the safest options are natural or well-tested medications. It should be noted that the human body's defense mechanisms react differently to viral and bacterial infections. The use of antiviral vaccines and antibiotic therapy against bacteria is common. This scientific study will present the issue of transmission of the bacterium Pseudomonas aeruginosa in places of religious worship, with the greatest emphasis on church objects that are constantly touched by the faithful.

Keywords: *Pseudomonas aeruginosa; Religious Artifacts; Infection; Bacteria; Silver Nanoparticles*

Introduction

The problem of emerging epidemics has accompanied the development of human civilization since its inception. Initially, people were unable to counteract the spreading epidemics. The first bacterial epidemic most harmful to humans was the Athenian epidemic, which occurred in the fifth century BC. It is believed that this was the first case of a plague epidemic recorded in the annals. As historical records indicate, the society of Athens was decimated, and those who were cured struggled with many diseases and even memory loss [1]. During the epidemic, there was a war between the Athenians and Sparta. Athens was a military power, and their goal was to conquer all of Greece. The accumulation of a significant number of people from the nearby regions of Athens in Athens was a favorable environment for the spread of infections [2]. Unfortunately, this also had an impact on the size of the army and significantly weakened the military power of Athens [3]. This source indicated that as much as over a quarter of the armed forces (1,050 out of 4,000 soldiers) sent to Potidae died as a result of plague infection. Another famous case of the plague was the plague of Justinian, which caused the death of almost one fifth of the population of the Mediterranean basin. Initially, however, based on historical reports, it was believed that Justinian's epidemic was the reason for over 40% of the

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population loss of the Byzantine Empire [4]. What deserves the greatest attention is the fact that the emission of this plague was so large that it reached several continents, including the western territories of present-day Poland [5]. Another well-documented bacterial plague was the Black Death from the period 1346 - 1353. According to historical reports, the Black Death was the cause of the death of nearly one third of the population of medieval Europe. It is even estimated that the population of Europe at that time was reduced by 60% [6]. It should be recognized that ignorance of the basics of hygiene, lack of awareness of the problem, and appropriate medicines were the main causes of the spread of the plague bacteria. According to the latest research, designed antibiotics effectively fight the plague bacterium.

However, it should be taken into account that this bacterium is becoming more and more resistant to antibiotics, which was found in the strain from Madagascar [7]. The above information is a historical fact that serves as a warning to subsequent generations and indicates that it is necessary to prevent bacterial mutations and their spread. As a result of careful observation and knowledge acquired over generations, people have learned basic hygiene rules, which has significantly reduced the emission capacity of viruses and bacteria. Initially, the main concern was the durability of food products, which, as a result of spoilage, were a source of numerous bacteria. A well-known and commonly used method of keeping food fresh was to place silver objects, mainly coins, in them. Sailors threw silver coins into jugs of water, which provided them with fresh water on hot days during a long voyage. There are numerous historical facts confirming that people were aware of the beneficial effects of silver but did not know how to process it properly. Current technology and knowledge make it possible to produce silver in various forms: coatings, layers, or colloids. Additionally, silver can be divided into fragments with nanometric dimensions and various shapes, even in the range of several nanometers. Many methods of producing nanosilver are known, divided into ionic [8] and non-ionic [9] silver, as well as methods such as chemical [10] or physical [11]. The ability to produce an appropriate and tested bactericidal preparation makes it possible to use it as a coating to protect against bacterial infection.

The cult of religious figures is widely known, and there are church treasures that are touched every day by believers from various parts of the world. The most vulnerable are those faithful who go to places of worship where the sacred treasure is kissed as a sign of appreciation. Nowadays, public transport is completely different than it was hundreds of years ago. Transferring bacteria currently takes as long as it takes to fly on an airplane. A distance of 10,000 km is now covered in just a dozen or so hours, which used to take many months. This means that a person with a bacterial infection without much indication of infection can be the source of an outbreak. Therefore, the ability to prevent the multiplication and spread of bacteria on church objects displayed every day in places of religious worship is important from the point of view not only of pilgrims but also of church authorities.

This paper will present the test results for the bacteria Pseudomonas aeruginosa exposed to nonionic colloidal nanosilver.

Experimental part

The bactericidal properties of the non-ionic silver solution were determined on the basis of microbial counts after the following procedure:

- Preparation of standard strains: 24-hour cultures of the standard strain with a concentration of 0.5 on the McFarland scale (0.5×10^8 CFU/ml, where CFU is colony-forming units) were used for the study. Ten-fold dilutions were performed—the tests used a suspension of strains with a density of approximately 104 CFU/mL.

- Samples of sterile PMMA (poly-methyl methacrylate) were placed on Petri dishes, and a suspension of the tested strains was placed on their surfaces in an amount of 50 µL per "base" (contact time of the suspension with PMMA was 15 minutes at room temperature).
- The tested samples were placed in 500 µl of PBS (buffered saline solution) with 0.5% r-r saponin in order to detach the adhered microorganisms;
- Incubation of Pseudomonas aeruginosa was carried out for 48 hours at 35°C, and then the microorganisms were counted.

A non-ionic silver preparation with a concentration of 50 ppm was used for the tests. Silver particles occur in the form of thin crystalline plates. Silver in the solution is in a non-ionic form, unlike the vast majority of ionic solutions available on the market. Solutions containing ionic silver produced by chemical methods are characterized by a much lower durability due to the higher reactivity of Ag particles. These particles combine with other elements, which negatively affects their bactericidal properties. The research used non-ionic silver produced by physical methods.

Results

Pseudomonas aeruginosa is a very dangerous bacterium that attacks, especially, people with reduced immunity. Tired and weakened people, such as pilgrims who often arrive at a place of religious worship after a long and exhausting journey, are particularly vulnerable to this type of bacteria. This bacterium is considered one of the most dangerous and difficult to combat [12]. Figure 1 shows the bacterium *Pseudomonas aeruginosa*.

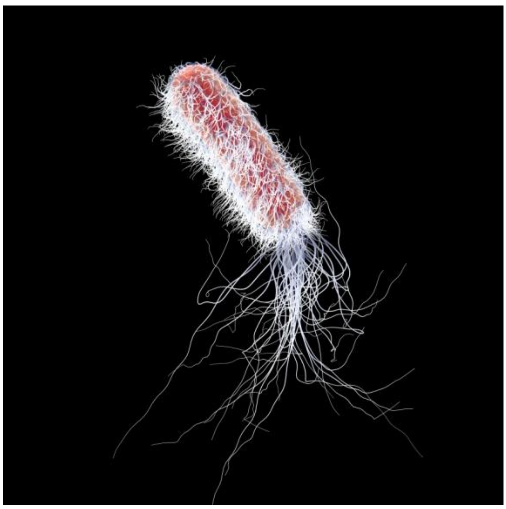

Fig. 1. *Pseudomonas aeruginosa* [12]

It is commonly believed that currently used antibiotics are a remedy for this bacterium. Unfortunately, research conducted, among others, in hospitals indicates that this bacterium becomes resistant to antibiotics particularly quickly [13]. *Pseudomonas aeruginosa* also has the ability to coexist and interact with a wide range of microorganisms, including bacteria, fungi, and viruses. Furthermore, the ability of *P. aeruginosa* to grow with other microorganisms is an important pathogenic feature with clinical significance [14] (Fig. 2).

Pseudomonas aeruginosa is capable of interacting and coexisting with a wide variety of microorganisms from different taxonomic groups, including bacteria, fungi, and viruses. These interactions play a critical role in its pathogenic potential, especially in clinical settings. The diagram illustrates significant interactions between *P. aeruginosa* and bacteria such as *Burkholderia cepacia, Staphylococcus aureus, Prevotella spp., Enterococcus faecalis, Streptococcus spp., Acinetobacter baumannii, Stenotrophomonas maltophilia, Veillonella spp., Actinomyces spp.*, and *Propionibacterium spp*. Additionally, its interactions with fungi such as *Aspergillus fumigatus* and *Candida albicans* and with viruses like respiratory syncytial virus (RSV), human rhinovirus, SARS-CoV-2, and influenza are depicted. Many of these microbial interactions occur in disease-related environments, highlighting the complex network that contributes to the persistence and virulence of *P. aeruginosa*.

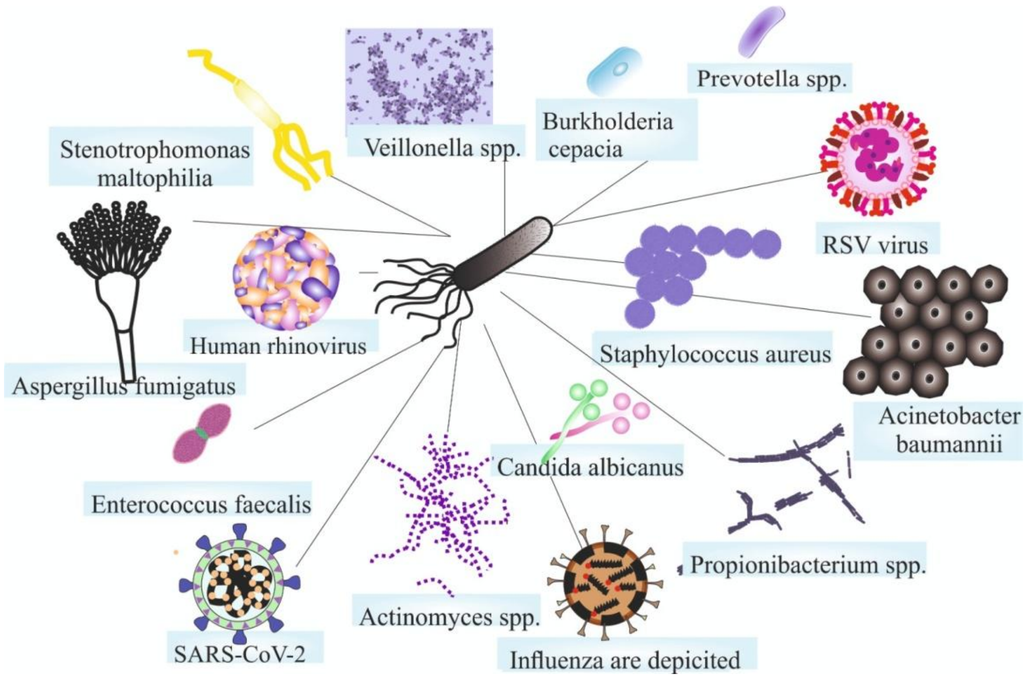

Fig. 2. Schematic representation of microbial interactions with *P. aeruginosa*

The most important stage of infection of this bacterium is the first stage, i.e., colonization. The considerations presented in this study will concern the process of colonization of *Pseudomonas aeruginosa* bacteria. It should be assumed that a small percentage of visitors to church relics are carriers of the *Pseudomonas aeruginosa* bacteria, and, under normal conditions, they are unlikely to be the source of a rapidly spreading epidemic. Unfortunately, if saliva with bacteria is applied to the jewelry, its transmission increases dramatically. In the case of particular relics, it is very likely that the bacteria will be transferred to other continents. People suffering from this bacterium will quickly be admitted to hospitals and will again contribute to the further development of this bacterium. This bacterium quickly settles in the human respiratory system and begins colonization. The work published in Signal Transduction and Targeted Therapy in

2022 shows that this bacterium attacks the lungs and disruptively blocks the treatment process [15].

Pseudomonas aeruginosa is a common pathogen found in various environments, including hospitals, where it can cause infections in virtually any organ. The bacterial lipopolysaccharides (LPS) trigger both TLR-4-dependent and -independent inflammatory responses in the lungs. Upon infection, epithelial cells release cytokines and chemokines, which lead to the recruitment of innate and adaptive immune cells. Neutrophil infiltration serves as an indicator of an activated inflammatory response. Although neutrophils are crucial for the host's defense mechanisms, their overactivation can lead to severe tissue damage, exacerbating the bacterial infection. Thus, understanding the balance between bacterial virulence factors and the host's immune response is key to developing effective treatment strategies for infections caused by *P. aeruginosa*.

Therefore, the best solution is to prevent the colonization of this bacterium, which blocks the next two stages of infection. The works [15, 17] indicate that many therapies are currently used against the *Pseudomonas aeruginosa* bacteria, including the use of nanomaterials in the form of silver particles.

The nanosilver used in the research is non-ionic silver produced by an explosive method [18]. Physically produced nanosilver is pure and has a characteristic flake shape, unusual for other production methods. Commonly produced nanocolloids are products containing silver particles in the form of microcrystals, spherical or cylindrical [19]. You should imagine what the contact surface is for a sphere and a cylinder: point and linear. This means that the action of the nanoparticle affects one point or line (Fig. 3).

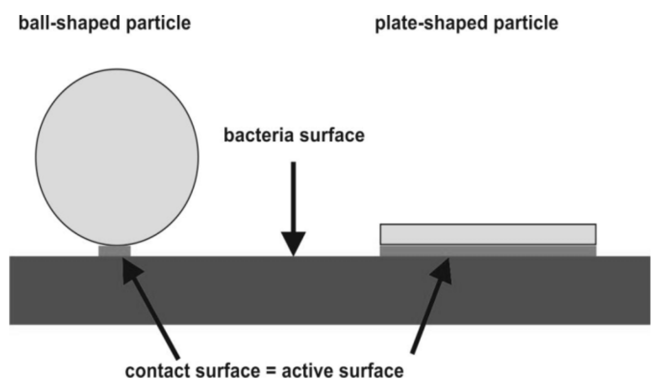

Fig. 3. Diagram of the difference in the action of ball-shaped and plate-shaped nanoparticles

In the case of a pad, the effect of the antibacterial particle covers a certain surface area. Additionally, the flakes can be overlapped, which allows them to cover large areas, creating a bactericidal active surface. The large surface area of the silver particles used in the form of flakes is their advantage over other shapes. Figure 4 shows a photo taken using a transmission electron microscope of a flat nanosilver particle produced by a physical method.

Assuming that the nanocolloid will be applied to the surface of the sacred object, a protective barrier will be obtained, constituting a coating of nanosilver. This coating will be antibacterial and will largely prevent the colonization of bacteria on the surface of the religious artifacts. The water will evaporate from the applied nanocolloid, and the silver nanoparticles will create an antibacterial coating. It is assumed that the nanocolloid will be applied in the form of a mist to the surface every 15 minutes. This treatment will make it possible to replenish antibacterial particles that will be wiped off by pilgrims. Scheme of the process for treating surfaces with nanosilver nanoparticles in the fogging process (Fig. 5).

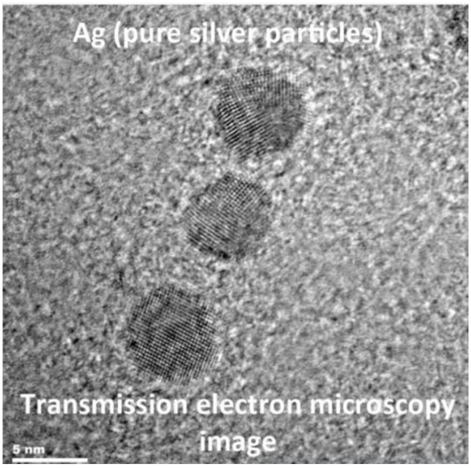

Fig. 4. Non-ionic silver nanoparticles produced by the explosive method. According to the information provided on the NanoKoloid

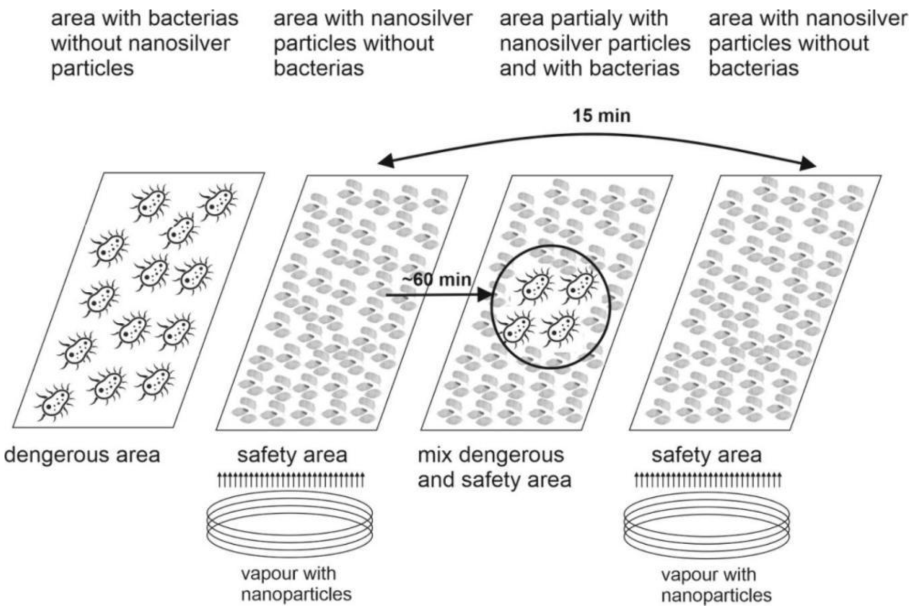

Fig. 5. Scheme of the process of replenishing non-ionic silver nanoparticles on the surface

The results of laboratory tests clearly indicate that the nanosilver used is well suited for use as a protective barrier in the process of colonization of *Pseudomonas aeruginosa* bacteria. Figure 6 shows the test results from the culture of *Pseudomonas aeruginosa* bacteria.

The CFU infective dose for the sterile pad was 420 CFU; approximately 300 CFU were recovered. In the case of a base coated with a silver solution with a concentration of 50 ppm, the infecting dose was 660 CFU. In this case, 48 colonies of *Pseudomonas aeruginosa* were counted. The experiment was performed 3 times; the data are presented in Table 1.

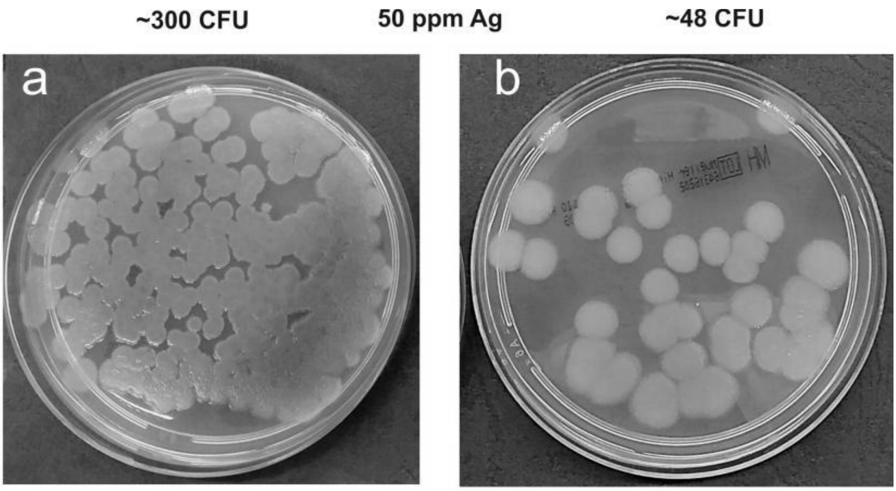

Fig. 6. Seeds for the suspension of the tested *Pseudomonas aeruginosa* strains for a) suspension recovered from the PMMA surface and b) suspension recovered from the PMMA surface after bathing in a 50 ppm silver solution

Table 1. Results of bactericidal tests obtained for sterile PMMA pads and those immersed in a silver solution with a concentration of 50 ppm

	Output density CFU/ml Average of 3 experiments	Amount of infecting dose CFU/substrate Average of 3 experiments	Number of recovered bacteria CFU/ substrate Average of 3 experiments	Ratio of microorganisms recovered
Pseudomonas aeruginosa on a sterile PMMA substrate	0.8×10^4	420 CFU	120 CFU	28.57%
Pseudomonas aeruginosa on a sterile PMMA substrate covered with a 50 ppm non-ionic silver solution	1.2×10^4	660 CFU	84 CFU	12.72%

The research shows that the use of a solution of silver nanoparticles reduced the growth of bacteria by more than half. It is worth noting that a solution with a very low concentration of 50 ppm was used. It is expected that by using slightly higher concentrations and by repeating the coating every 15 minutes, it will be possible to completely eliminate the risk of bacteria on the religious artifacts.

Discussion

Taking up the issue of protecting people against bacterial infection is a very important issue nowadays when antibiotics are slowly starting to lose their potency. The human body is increasingly weakened by the introduction of antibiotic therapies. Particularly weakened people can easily adopt the *Pseudomonas aeruginosa* bacteria, which will begin the colonization stage in a weak body. Religious places where pilgrims come from all over the world are very exposed to concentrations of people carrying various strains of bacteria. As mentioned earlier, the *Pseudomonas aeruginosa* bacterium is viral and bacteriological, which makes it very dangerous. Additionally, often in tight, cool, and dark sanctuaries there are relics that are touched and even kissed by the faithful for veneration. In such places it is very easy to leave bacteria that can survive on the surface of the religious artifacts for many hours or even days. Based on the laboratory tests carried out, it can be clearly stated that the use of the latest achievements in physics and nanotechnology favors the protection of church monuments while maintaining the safety of the faithful in the form of a protective shield composed of non-ionic silver nanoparticles in the form of flakes with a size of several nanometers. The shape, quality, and size of the nanoparticle itself are crucial.

Conclusions

The use of the method of applying an antibacterial coating made of silver colloid is a good solution that may in the future influence the constantly growing popularity of visiting church relics. It is difficult to imagine nowadays that information about sudden bacterial infections will not be quickly disseminated in the media. There can only be one effect of this: concern for your health. Faced with the possibility of getting sick, people will try to prevent and combat any contact with the source of the disease. This may lead to the limitation or complete exclusion of the possibility of visiting places where holy relics are located. This state of affairs will be very difficult for part of society to understand and will constitute a pretext for religious incitement. Therefore, the latest issues related to materials engineering can provide a solution to these types of problems. Coatings made of non-ionic silver will largely protect visitors to church relics against bacterial infections, in particular by the difficult-to-fight Pseudomonas bacteria. Nanotechnology provides many opportunities and should be used to bring as many benefits to humanity as possible.

Acknowledgments

The authors would like to thank Nanokoloid for providing the TEM microscope image.

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Received: December 20, 2025
Accepted: May 15, 2026