



In vitro antimicrobial activity of silver nanoparticles against selected Gram-negative and Gram-positive pathogens

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Abstract

Background and aim. Infections caused by pathogenic bacteria increase patient morbidity and mortality and significantly raise treatment costs. The use of silver nanoparticles as an alternative treatment for *S aureus*, *E coli*, *MRSA*, *E faecalis*, *K pneumoniae* and *P aeruginosa* indicates their antibacterial effect and prompts medical research to consider the next generation of antibacterial drugs that could change antibiotic therapy. By combining silver nanoparticles with different classes of antibiotics, the antibacterial effect is evidenced by increased values of the inhibition zone compared to the values obtained for some antibiotics commonly used in the treatment of bacterial infections. This study focuses on comparing the antibacterial activity of antibiotics versus antibiotics combined with silver nanoparticles against various bacteria, by comparing inhibition zones obtained for both. We aim to prove that the size of the inhibition zone for antibiotics combined with silver nanoparticles is greater, thus confirming the improved antibacterial effect.

Methods. In this study we tested the antibacterial activity of solutions of silver nanoparticles alone or in combination with different antibiotics. We used standard bacterial strains, ATCC, both Gram positive bacteria *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, as well as Gram negative bacteria *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, but also on clinical isolates: a strain *MRSA* (*Methicillin Resistant Staphylococcus aureus*) and a PDR strain (pan drug resistant) of *Klebsiella pneumoniae*. Bacterial identification was performed using Vitek MS analyzer (bioMerieux). Antibiotic susceptibility determination was performed with VITEK2 COMPACT SYSTEM (bio Merieux, Inc Durham NC) with ready to use VITEK AST cards. The interpretation of the results was done in compliance with EUCAST 2023-2024 standards. Testing was performed for several classes of antibiotics, silver nanoparticle solutions in 2 concentrations (10 µg/mL and 100 µg/mL) and for combinations of antibiotics with silver nanoparticle solutions. The diameter of the inhibition zone (ZOI) for silver nanoparticles, antibiotics and silver nanoparticles combined with antibiotic against each bacterium was expressed in millimeters. The Kirby-Bauer disk-diffusion method, in accordance with current EUCAST standards, was used to analyze the antibacterial effect of antibiotics, silver nanoparticles, and antibiotics combined with silver nanoparticles at biocompatible doses of 10 and 100 µg/mL. The experiments were conducted in triplicate, and the results were almost identical.

Results. The results of this study show that the silver nanoparticles displayed antibacterial activity, proven by the appearance of the inhibition zone, in various sizes, for all bacteria studied. The antibiotic classes tested were beta-lactams, first, second, third and fourth generation cephalosporins, macrolides, fluoroquinolones, lincosamides, aminoglycosides, glycopeptides, tetracyclines, oxazolidinones, sulfonamides, rifamycins, amphenicols. Testing *S aureus* ATCC 29213, the highest zone of inhibition was demonstrated for cephalosporins (32.6667 ± 0.701 mm), macrolides (31.6667 ± 0.701 mm, and lincosamides (29.6667 ± 0.701 mm). Testing *MRSA* (internal code GR0333), the highest zone of inhibition for combination of

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silver nanoparticles and antibiotics was demonstrated for fluoroquinolones (36.3333 ± 0.701 mm), lincosamides (32.3333 ± 0.701 mm), Fusid acid (32.3333 ± 0.701 mm) and aminoglycosides (31.3333 ± 0.701 mm). Testing *E coli* ATCC 25922 the highest zone of inhibition was for Fosfomycine, 39 mm and for *E faecalis* ATCC 29212 for aminoglycosides was 19 mm. For *K pneumoniae* (internal code GQ8575) the inhibition zone for silver nanoparticles 100 $\mu\text{g/mL}$ was 12.3333 ± 0.701 mm and for *P aeruginosa* ATCC 27253 was 16 ± 1.214 mm.

Conclusions. The use of metallic nanoparticles, especially silver ones, as antimicrobial agents with definite bactericidal activity has led medical specialists to consider this new treatment which may change antibacterial therapy. Studies of in vitro combinations between silver nanoparticles and different classes of antibiotics represent a highly efficient and effective new antibacterial treatment against multidrug-resistant bacteria. To avoid the problem of antimicrobial resistance associated with conventional antibiotics, it is necessary to understand the adaptive mechanisms of bacteria under the action of metal nanoparticles, which could be exploited in future studies. Further in vitro and in vivo studies that would assess specify the biocompatibility and toxicity of silver nanoparticles will make these super nanomaterials the medicines of the future.

Keywords: silver nanoparticles, antibacterial effect, *Staphylococcus aureus*, *Escherichia coli*, antibiotic treatment

Background and aim

Antibiotics are one of the most important discoveries in the medical field for treating infections caused by pathogenic bacteria. The discovery of penicillin in 1928 by Alexandre Fleming led to a significant decrease in the number of deaths from infections. It paved the way for the discovery of other classes of antibiotics capable of fighting infections [1]. The unjustified use of antibiotics, self-medication, overdose, and poor socio-economic conditions have led to the emergence of microbial resistance and multidrug-resistant bacteria [2]. Nosocomial infections affect patients worldwide. They represent a serious public health problem, generating huge costs for health systems, and cause a significant increase in the number of associated diseases and deaths among patients [3,4].

In the article published in 2023, Dove et al show that in 2019 in the US, over 2.8 million infections caused by multiresistant germs were reported, which led to the death of over 35,000 patients. Globally, in the same year, over 1.27 million deaths had the same cause [5].

In the article published in 2022, Masimen et al show that the bacteria causing the most deaths are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [1].

Nanotechnology has emerged as a branch of science that synthesizes very small particles to combat bacterial infections which are difficult to treat using traditional means. Among the gold, copper, titanium, platinum, zinc, magnesium nanoparticle, the most studied are silver nanoparticles due

to their special properties that make them highly effective in the medical use cases, among other industries [6-9]. The most important properties of silver nanoparticles are their antimicrobial, antitumor, antiviral, antifungal and anti-inflammatory effects. Silver nanoparticles can be produced by several methods, physical, chemical, and biological, each with its advantages and disadvantages. Physical methods require expensive equipment, large amounts of energy, and special temperature and pressure conditions. Chemical methods are toxic and harmful to humans, animals, and the environment. Biological methods are the preferred method because they are environmentally friendly, do not require toxic chemicals, special temperature and pressure conditions or any special equipment [1,4,10-19].

The activity of silver nanoparticles on Gram positive and Gram negative bacteria is mainly influenced by their size, shape and electrical charge. Studies have shown that small, spherical nanoparticles have a much more intense antibacterial effect compared to larger ones and other shapes [14,20-23].

Gram negative bacteria are more susceptible to silver nanoparticles compared to gram positive ones, due to the different structure of the bacterial wall. They have a thin peptidoglycan layer with an additional outer membrane formed by lipopolysaccharides.

Gram positive bacteria have a thicker peptidoglycan layer. This difference in thickness makes it easier for positive silver ions to enter inside the Gram negative bacterial cell [24,25].

Although the mechanism of the antibacterial action is not fully elucidated, the antibacterial activity is obtained by the interaction between the cell membrane, which is negatively charged, and silver ions, which are positively charged. This interaction destroys the bacterial cell membrane, followed by the production of oxidative stress and reactive oxygen species. Bacterial cell death occurs due to the disruption of ATP synthesis and cellular metabolism. It is followed by the inhibition of nutrition, the change of gene expression and blocking of the respiration process at the cell level [11,26,27].

The antibacterial effect of silver nanoparticles can be further enhanced by combining them with various antibiotics, making them useful especially against multidrug-resistant bacteria [2,28]. Studies demonstrate that lower doses of antibiotics are required in these combinations, and the negative side effects of antibiotics combined with nanoparticles are diminished [14]. The nanoparticles also demonstrate toxic effects on human and animal cells depending on the concentrations used, route and duration of exposure. Additionally, these effects are proven by both in vitro and in vivo studies, even if the mechanisms of action are not fully elucidated [1,7,10,18,29,30].

The emergence of multiresistant bacteria requires increasingly complex antibiotic treatments for longer periods, that increase the toxicity on the human body. All antibiotics used in the therapy of infections can have various undesirable side effects. Therefore, the discovery of other combinations of effective antibacterial substances with fewer side effects is imperative. For this reason, silver nanoparticles may become a viable treatment alternative soon.

This study can be a start for further investigations aimed at developing new treatments with silver nanoparticles combined with antibiotics. These new drugs can be used to treat infections caused by multidrug-resistant bacteria that are difficult to combat with classical antibiotics.

This study focuses on comparing the antibacterial activity of antibiotics versus antibiotics combined with silver nanoparticles against various bacteria, by comparing inhibition zones obtained for both.

We aim to prove that the size of the inhibition zone for antibiotics combined with silver nanoparticles is greater, thus confirming the improved antibacterial effect.

Methods

Given the hypothesis that bacterial infections negatively influence the evolution of a patient, we conducted an in vitro study. We tested the antibacterial activity of solutions of silver nanoparticles (in two concentrations), alone or in combination with different antibiotics. We used standard bacterial strains ATCC, both Gram positive bacteria *Staphylococcus aureus*

ATCC 29213, *Enterococcus faecalis* ATCC 29212, as well as Gram negative bacteria *Escherichia coli* ATCC 25922, *Pseudomonasaeruginosa* ATCC 27853, but also on clinical isolates: a strain MRSA (*Methicillin Resistant Staphylococcus aureus*) and a PDR strain (pan drug resistant) of *Klebsiella pneumoniae*.

We have studied standard ATCC strains and two strains that were isolated from patients which are part of the collection of IRGH O Fodor Cluj-Napoca Microbiology Laboratory. The Gram positive bacteria used are *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212. The Gram negative bacteria used are *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

The first strain isolated from patients is a strain of MRSA (*Methicillin Resistant Staphylococcus aureus*, (internal code GR 0333), resistant to penicillin, penicillin+beta lactamase inhibitor, first, second, third and fourth generation cephalosporins, fluoroquinolones, aminoglycosides, carbapenems, macrolides and tetracyclines. The second isolated strain is a PDR (PANdrug resistant) strain of *Klebsiella pneumoniae* (internal code GQ 8575), resistant to: betalactams (including carbapenems), fluoroquinolones, aminoglycosides, colistin, trimethoprim+sulfamethoxazole and first, second, third and fourth generation cephalosporins.

The silver nanoparticles were purchased from Sigma-Aldrich (St. Louis, MO, USA). They have a purity of 99.99%, a spherical shape and a size of 2 nm. They were stored in dark glass containers at room temperature.

A bacterial suspension of turbidity 0.5 McFarland (corresponding to 1.5×10^8 CFU/mL) was made for each isolate from colonies isolated for 24 h bacterial culture on Columbia agar plates + 5% sheep blood (BioMerieux). The colonies were sown in cloth on Mueller-Hinton agar plates (BioMerieux). After sowing, sterile wells were made, into which solutions of silver nanoparticles were introduced and combinations of antibiotics with solutions of silver nanoparticles were introduced.

From colonies isolated for 24 h bacterial culture on Columbia agar plates + 5% sheep blood (BioMerieux), bacterial identification was performed using Vitek MS analyzer (bioMerieux). Antibiotic susceptibility determination was performed with VITEK2 COMPACT SYSTEM (bio Merieux, Inc Durham NC) with ready to use VITEK AST cards.

The interpretation of the results was done in compliance with EUCAST 2023-2024 (European Committee on Antimicrobial 2023-2024) standards.

Testing was performed for several classes of antibiotics, silver nanoparticle solutions in 2 concentrations (10 µg/mL and 100 µg/mL) and for combinations of antibiotics with silver nanoparticle solutions.

Wells of approximately 8 mm were made with a 500 µL pipette tip, under aseptic conditions. Using a

micropipette, we pipetted 100 μ L into each well. The plates were incubated at 37°C for 24 hours.

The diameter of the inhibition zone (ZOI) for silver nanoparticles, antibiotics and silver nanoparticles combined with antibiotic against each bacterium was expressed in millimeters and measured using a graduated ruler.

The absence of the zone of inhibition indicated a lack of antimicrobial activity. The Kirby-Bauer disk-diffusion method, in accordance with current EUCAST standards, was used to analyze the antibacterial effect of antibiotics, silver nanoparticles, and antibiotics combined with silver nanoparticles at biocompatible doses of 10 and 100 μ g/mL.

To evaluate the bactericidal effect, micro tablets for antimicrobial susceptibility test, with a standard diameter of 6 mm Oxoid, ready for use with or without silver nanoparticles, which were placed in plates with Mueller Hinton culture media (MH) seeded with bacteria.

The experiments were conducted in triplicate, and the results were almost identical.

The entire process was carried out using a Class II microbiological hood fitted with laminar flow to avoid any contamination during the experiment.

The antibiotic classes tested were beta-lactams, first, second, third and fourth generation cephalosporins, macrolides, fluoroquinolones, lincosamides, aminoglycosides, glycopeptides, tetracyclines, oxazolidinones, sulfonamides, rifamycins, amphenicols. The antibiotics tested were Penicillin, Cefotaxim, Erythromycin, Clindamycin, Gentamicin, Tobramycin, Amikacin, Ciprofloxacin, Levofloxacin, Norfloxacin, Tetracycline, Biseptol, Linezolid, Vancomycin, Teicoplanin, Ampicillin, Imipenem, Meropenem, Cefepim, Ticarcillin, Ceftazidime Ampicillin, Amoxicillin, Ticarcillin, Fosfomycin and Fusidic Acid.

Results

In this study, the antibacterial activity of silver nanoparticles was tested against several bacteria: *S aureus* ATCC 29213, MRSA (internal code GR 0333), *E faecalis* ATCC29212, *E coli* ATCC 25922, *K pneumoniae* (internal code GQ 8575) and *P aeruginosa* ATCC 27853.

The results of this study show that the silver nanoparticles displayed antibacterial activity, proven by the appearance of the inhibition zone, in various sizes, for all bacteria studied. The amount of silver nanoparticle solution applied to the culture medium plate determines the antibacterial effect of silver nanoparticles, therefore the area of inhibition was higher when applying 100 μ L compared to applying a much smaller amount of solution, i.e. 20 μ L. If 200 μ L of silver nanoparticle solution is applied to well No 4, the overflow solution produces a total inhibition of growth of the tested bacterial strain throughout the overflowing area (Figure 1).



Figure 1. Influence of the amount of silver nanoparticle solution on the culture plate when testing *S aureus*.

By testing *S aureus* ATCC 29213 for several classes of antibiotics (Table I) we obtained different sizes of measured areas of inhibition. In the case of *S aureus* ATCC 29213 testing for beta-lactams, cephalosporins, macrolides and lincosamides (Figure 2) the highest inhibition zone was obtained in the case of Cefoxitin (29.3333 ± 0.701 mm) 2nd generation cephalosporin, followed by Clindamycin (26.6667 ± 0.701 mm), antibiotic of the lincosamide class and then of Erythromycin (25.6667 ± 0.701 mm) of the macrolide class. Penicillin had the smallest area of inhibition of just 14.3333 ± 0.701 mm of the antibiotics tested on this plate (Figure 2, Figure 8).

In the case of testing *S aureus* ATCC 29213 with the aminoglycoside class of antibiotics, (Figure 3, Figure 4) in all 3 antibiotics tested (gentamicin, tobramycin and amikacin) the inhibition zone was approximately equal, with values between 20 and 21 mm diameter (20.3333 ± 0.701 mm and 21.3333 ± 0.701 mm).

In the case of fluoroquinolones (Figure 5), we obtained almost identical results for all 3 antibiotics tested (Ciprofloxacin, Levofloxacin and Norfloxacin).

Areas of inhibition over 25 mm were obtained in the case of antibiotic class testing of tetracyclines, sulfonamides, oxazolidinones (Figure 6), Fusidic Acid (Figure 7), ansamycins and amphenicols.

In the case of *S aureus* ATCC 29213 testing with penicillins and cephalosporins of the 3rd generation, (Figure 9) the largest area of inhibition was obtained by Cefotaxim, 24 mm and in the case of Penicillin we obtained 14.3333 ± 0.701 mm.

Inhibition zone sizes below 16 mm were obtained in the case of *S aureus* ATCC 29213 testing for glycopeptides (Figure 10), respectively Vancomycin 14.3333 ± 0.701 mm and 15.333 ± 0.701 mm in the case of Teicoplanin.

Table I. Testing of *S aureus* ATCC 29213 with various antibiotics and the obtained zone of inhibition (mm±Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm±SD) |
|----------------------------------|-----------------------|----------------------------|
| Beta-lactamins | Penicillin 1 µg | 14.3333 ± 0.701 |
| Cephalosporins second generation | Cefoxitin 30 µg | 29.3333 ± 0.701 |
| Cephalosporins third generation | Cefotaxim 30 µg | 24.3333± 0.701 |
| Macrolides | Erythromycin 15 µg | 25.6667 ± 0.701 |
| Lincosamides | Clindamycin 2 µg | 26.3333 ± 0.701 |
| Aminoglycosides | Gentamicin 10 µg | 21.3333 ± 0.701 |
| | Tobramycin 10 µg | 20.3333 ± 0.701 |
| | Amikacin 30 µg | 20.3333 ± 0.701 |
| Fluoroquinolones | Ciprofloxacin 5 µg | 23.3333 ± 0.701 |
| | Levofloxacin 5 µg | 24.3333 ± 0.701 |
| | Norfloxacin 10 µg | 23.3333 ± 0.701 |
| Tetracyclines | Tetracycline 30 µg | 27.6667 ± 2.804 |
| Sulfonamides | Bisepitol 25 µg | 29.6667 ± 3.056 |
| Oxazolidinones | Linezolid 10 µg | 24.3333 ± 0.701 |
| Fusidic Acid | FusidicAcid 10 µg | 30.3333 ± 0.701 |
| Glycopeptides | Vancomycin 5 µg | 14.3333 ± 0.701 |
| | Teicoplanin 30 µg | 15.3333 ± 0.701 |
| Ansamycins | Rifampicin 5 µg | 30.3333 ± 0.701 |
| Amphenicols | Chloramphenicol 30 µg | 28.3333 ± 0.701 |

Table II. Testing of *S aureus* ATCC 29213 with various antibiotics combined with silver nanoparticles 10 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation).

| Antibiotic class | Antibiotic and silver nanoparticles | Zone of Inhibition (mm±SD) |
|------------------|-------------------------------------|----------------------------|
| Beta-lactamins | Penicillin 1 µg + AgNps 10 µg/mL | 14.6667 ± 2.804 |
| Cephalosporins | Cefoxitin 30 µg + AgNps 10 µg/mL | 32.6667 ± 0.701 |
| | Cefotaxim 30 µg + AgNps 10 µg/mL | 25.6667 ± 0.701 |
| Macrolides | Erythromycin 15 µg+ AgNps 10 µg/mL | 26.6667 ± 0.701 |
| | Erythromycin 15 µg+ AgNps 100 µg/mL | 31.6667 ± 0.701 |
| Lincosamides | Clindamycin 2 µg+ AgNps 10 µg/mL | 29.6667 ± 0.701 |
| | Clindamycin 2 µg+ AgNps 100 µg/mL | 29.6667 ± 0.701 |
| Aminoglycosides | Gentamicin 10 µg + AgNps 10 µg/mL | 22.6667 ± 0.701 |
| | Tobramycin 10 µg + AgNps 10 µg/mL | 21.6667 ± 0.701 |
| | Amikacin 30 µg + AgNps 10 µg/mL | 20.6667 ± 0.701 |
| Fluoroquinolones | Ciprofloxacin 5 µg+ AgNps 10 µg/mL | 24.6667 ± 0.701 |
| | Levofloxacin 5 µg + AgNps 10 µg/mL | 26.6667 ± 0.701 |
| | Norfloxacin 10 µg+ AgNps 10 µg/mL | 26.6667 ± 0.701 |

When testing *S aureus* ATCC 29213 with other classes of antibiotics (Figure 11) (Tetracycline, Rifampicin and Chloramphenicol) we obtained elevated values, over 27 mm of the inhibition zone for all 3 antibiotics tested.

The highest areas of inhibition were obtained when testing for Fusidic Acid 30.3333 ± 0.701 mm (Figure 12).

In the case of betalactamines we tested Penicillin 1 µg and obtained an inhibition zone of 14.333 ± 0.701 mm, in the case of second-generation cephalosporins (Figure 13) – Cefoxitin 30 µg we obtained 29.3333 ± 0.701 mm.

In the case of testing *S aureus* ATCC 29213 in combinations of antibiotics with silver nanoparticles with a concentration of 10 µg/ml (Table II), the highest areas of

inhibition were obtained in the case of second-generation cephalosporins - Cefoxitin (Figure 13), 32.6667 ± 0.701 mm, in the case of macrolides (Figure 8), Erythromycin 31.6667 ± 0.701 mm and lincosamides (Figure 8), Clindamycin 29.6667 ± 0.701 mm. Good results were also obtained in the case of fluoriquinolones testing (Figure 5), (Ciprofloxacin, Levofloxacin and Norfloxacin) respectively 24.6667 ± 0.701 mm, 26.6667 ± 0.701 mm. The lowest value of the inhibition zone was obtained in the test of *S aureus* ATCC 29213 with beta-lactamine and silver nanoparticles with a concentration of 10 µg/ml (Figure 13), Penicillin 1 µg 14.6667 ± 0.701 mm.

In the case of *S aureus* ATCC 29213 testing with 3rd

generation cephalosporins -Cefotaxime 30 µg combined with silver nanoparticles 10 µg/ml, the inhibition zone was less than 25.6667 ± 0.701 mm compared to second-generation cephalosporins that obtained a zone of inhibition greater of about 7 mm (32.6667 ± 0.701 mm) (Figure 9), (Table II).

When testing *S aureus* ATCC 29213 with glycopeptides, Fusidic Acid and silver nanoparticles) we obtained a high inhibition zone only in the case of Fusidic Acid, 28 mm and in the case of silver nanoparticles 100 µg/mL, the inhibition zone was 13 mm.

Testing of *S aureus* ATCC 29213 with a solution of the antibiotic class of beta-lactams, macrolides, lincosamides and silver nanoparticles with a concentration of 10 µg/mL was higher. The 100 µg/mL silver nanoparticles solution had a higher bactericidal effect than 10 µg/mL. The combination of these and the 10 µg/mL silver nanoparticles solution proved equally effective (Figure 2). We did not achieve synergistic effect between the antibiotics used and the silver nanoparticle solution in both concentrations.

When testing *S aureus* ATCC 29213 on silver nanoparticles with concentrations of 10 and 100 µg/ml, we obtained values of the inhibition zone between 9 and 15 mm diameter (9.3 ± 1.034 mm and 14 ± 1.577 mm), (Table III). For combinations of 10 µg/mL silver nanoparticles and the tested antibiotics, the size of the inhibition zone was 1-2 mm larger. The same values of the inhibition were obtained, but we did not achieve a synergistic effect between the silver nanoparticle solution and the antibiotic discs. The inhibition zone was higher for the 100 µg/mL silver nanoparticle solution, i.e. 13 mm, compared to the 10 µg/mL solution, which was 11 mm. Using the same antibiotics for testing, the antibiotic inhibition zones were the same as with the first plate, but the more concentrated silver nanoparticles solution of 100 µg/mL developed a larger inhibition zone compared to the more diluted 10 µg/mL solution.

When testing *S aureus* ATCC 29213 on penicillins, second generation cephalosporins, glycopeptides and silver nanoparticles we obtained the highest inhibition zone for silver nanoparticles 100 µg/mL, 16 mm, proving that in this case, silver nanoparticles are effective as a bactericidal agent.

Table III. Testing of *S aureus* ATCC 29213 with various antibiotics combined with silver nanoparticles 10 µg/mL and 100 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation).

| Silver nanoparticles | Zone of Inhibition (mm±SD) |
|----------------------|----------------------------|
| AgNps 10 µg/mL | 9.3 ± 1.034 |
| AgNps 100 µg/mL | 14 ± 1.577 |

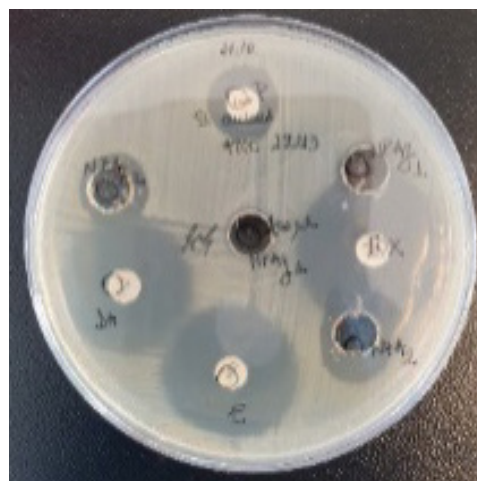


Figure 2. Testing *S aureus* to beta-lactams, macrolides, lincosamides and silver nanoparticles and combinations.

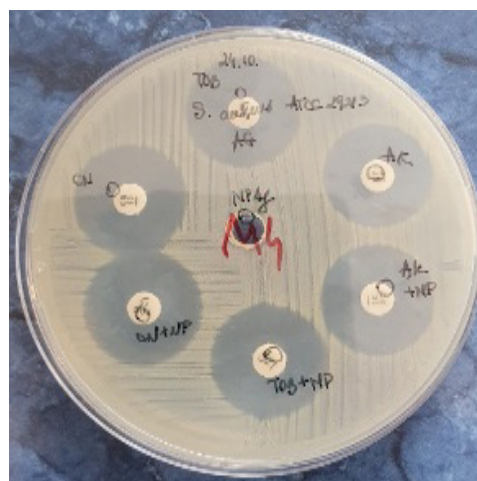


Figure 3. Testing *S aureus* to aminoglycosides, silver nanoparticles and their combinations.

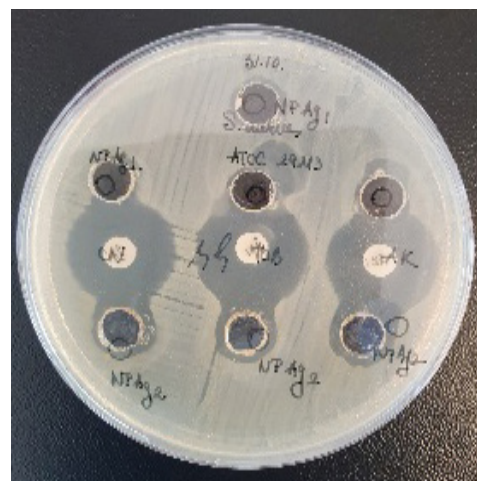


Figure 4. Testing *S aureus* to aminoglycosides and silver nanoparticles of 10 and 100 µg/mL.

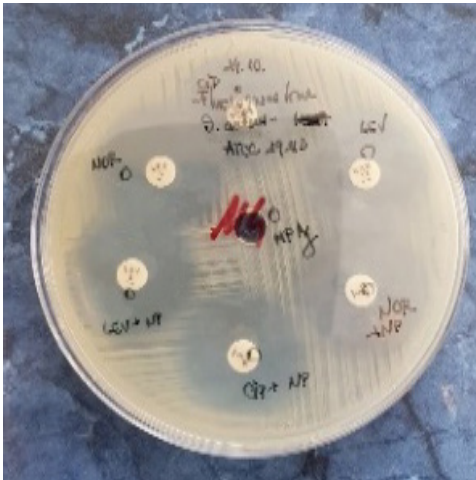


Figure 5. Testing *S. aureus* to fluoroquinolones, 10 µg/mL silver nanoparticles and their combinations.

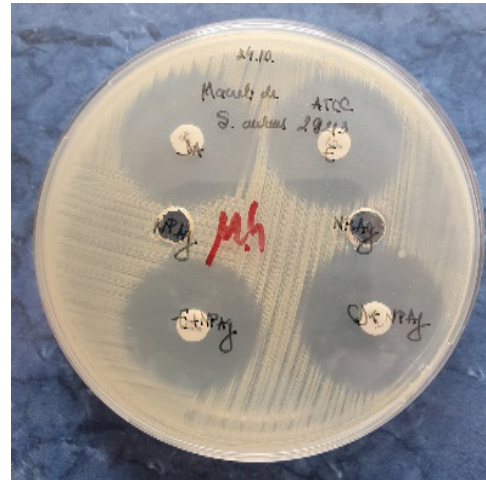


Figure 8. Testing *S. aureus* to macrolides, lincosamides and combinations with silver nanoparticles.

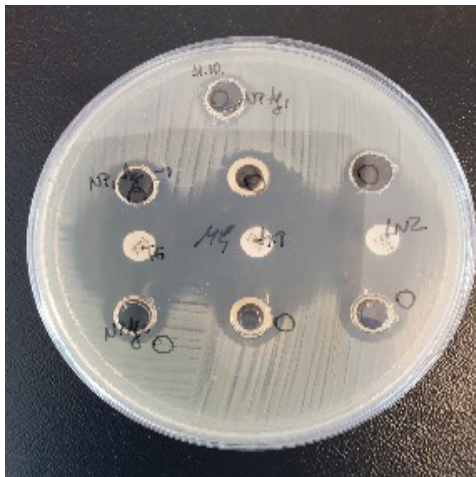


Figure 6. Testing *S. aureus* to tetracyclines, sulfonamides, oxazolidinones and silver nanoparticles.

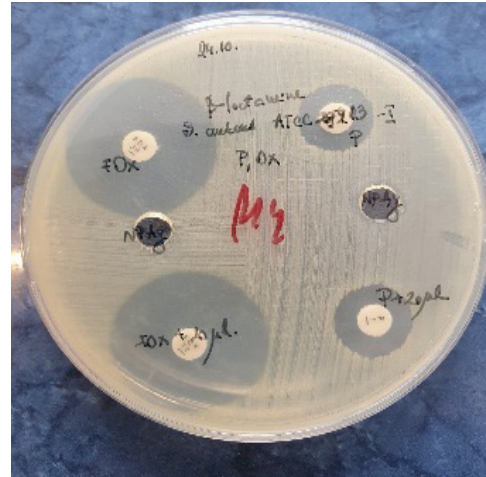


Figure 9. Testing *S. aureus* to penicillins, third generation cephalosporins and silver nanoparticles.

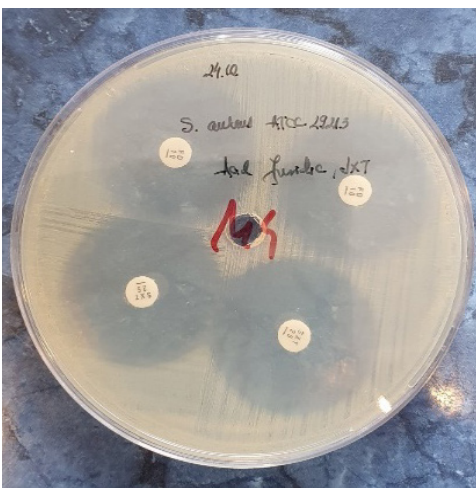


Figure 7. Testing *S. aureus* to Bisepitol and Fusidic Acid.

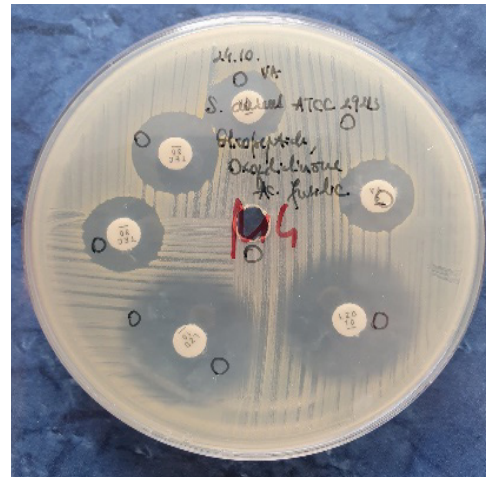


Figure 10. Testing *S. aureus* to oxazolidinones, glycopeptides and silver nanoparticles.

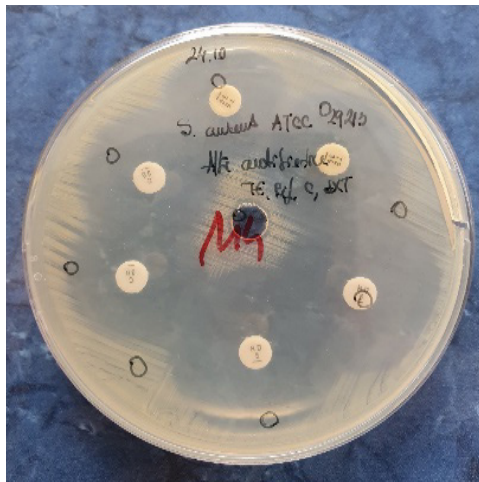


Figure 11. Testing *St aureus* to other antibiotics and silver nanoparticles.

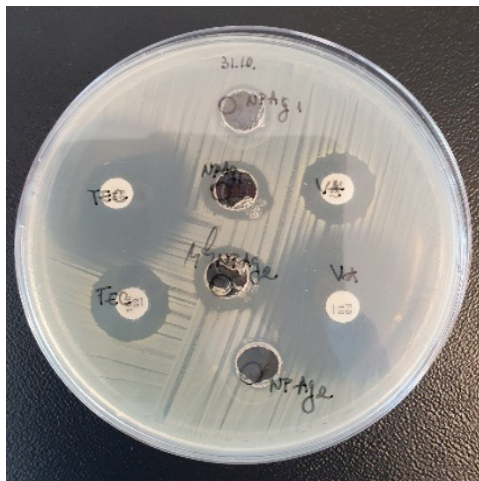


Figure 12. Testing *S aureus* to glycopeptides, Fusidic acid and silver nanoparticles.

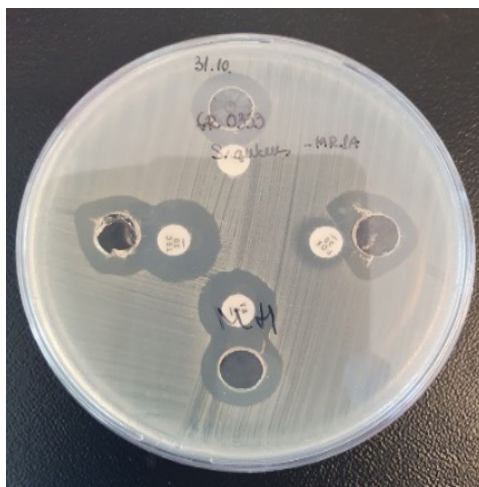


Figure 13. Testing *S aureus* to penicillins, 2nd generation cephalosporins, glycopeptides and silver nanoparticles.

In the case of MRSA (internal code GR 0333) (Table IV), the area of inhibition was much lower in the Penicillin 1 µg ($6.3333 \pm 0,701$ mm) test and only 12.3333 ± 0.701 mm in the Cefotaxime 30 µg test (Figure 14).

In MRSA (internal code GR 0333) testing for macrolides and lincosamides (Figure 15), the inhibition zones obtained were small for Erythromycin (12.3333 ± 0.701 mm) and 30.3333 ± 0.701 mm for Clindamycin (Table IV).

In MRSA (internal code GR 0333) testing for aminoglycosides (Figure 16), we obtained increased inhibition zones for all antibiotics of this class of about 30 mm respectively 29.3333 ± 0.701 mm for Gentamicin, 31.3333 ± 0.701 mm for Tobramycin and 30.3333 ± 0.701 mm for Amikacin (Table IV).

In MRSA (internal code GR 0333) testing for fluoroquinolones (Figure 17), we obtained increased inhibition zones for all antibiotics of this class tested: Ciprofloxacin 33.3333 ± 0.701 mm, Levofloxacin 36.3333 ± 0.701 mm and Norfloxacin 31.3333 ± 0.701 mm.

When testing MRSA (internal code GR 0333) for other antibiotics (Rifampicin, Tetracycline and Chloramphenicol) (Figure 18), we obtained areas of inhibition ranging from 10.3333 ± 0.701 mm, for Tetracycline to 25.3333 ± 0.701 mm for Rifampicin (Table IV).

MRSA (internal code GR 0333) testing for glycopeptides and oxazolidinones (Figure 19), showed antibiotics in these classes to be effective and have developed large areas of inhibition (Table IV).

MRSA (internal code GR 0333) testing with penicillins, glycopeptides, second-generation cephalosporins (Figure 20), yielded inhibition zones above 15 mm for glycopeptides and 12 mm for second-generation cephalosporins. Penicillin developed the smallest area of inhibition (6.3333 ± 0.701 mm) on this culture plate (Table IV). When testing MRSA (internal code GR 0333) with Bisseptol and Fusidic Acid (Figure 21), we obtained values above 30 mm of inhibition zone for the both antibiotics tested.

Combining the antibiotics tested with the 10 µg/mL silver nanoparticle we obtained the same inhibitory effect as the antibiotic.

For fluoroquinolones combined with silver nanoparticles at a concentration of 10 µg/ml, the size of the inhibition zone was identical to that obtained in MRSA (internal code GR 0333) testing for antibiotics of this class alone, proving that the low concentration of silver nanoparticles exhibits low inhibitory effect (Table V). Nor did the other combinations of antibiotics with low-concentration silver nanoparticles show greater bactericidal effect compared to the bactericidal effect of the antibiotic.

Table IV. Testing of *MRSA* (internal code GR 0333), with various antibiotics and the obtained zone of inhibition (mm± Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm±SD) |
|------------------|-----------------------|----------------------------|
| Beta-lactamins | Penicillin 1 µg | 6.3333 ± 0.701 |
| Cephalosporins | Cefoxitin 30 µg | 12.3333 ± 0.701 |
| | Cefotaxim 30 µg | 12.3333 ± 0.701 |
| Macrolides | Erythromycin 15 µg | 12.3333 ± 0.701 |
| Lincosamides | Clindamycin 2 µg | 30.3333 ± 0.701 |
| Aminoglycosides | Gentamicin 10 µg | 29.3333 ± 0.701 |
| | Tobramycin 10 µg | 31.3333 ± 0.701 |
| | Amikacin 30 µg | 30.3333 ± 0.701 |
| Fluoroquinolones | Ciprofloxacin 5 µg | 33.3333 ± 0.701 |
| | Levofloxacin 5 µg | 36.3333 ± 0.701 |
| | Norfloxacin 10 µg | 31.3333 ± 0.701 |
| Tetracyclines | Tetracycline 30 µg | 10.3333 ± 0.701 |
| Sulfonamides | Biseptol 25 µg | 30.3333 ± 0.701 |
| Oxazolidinones | Linezolid 10 µg | 28.3333 ± 0.701 |
| | Fusidic Acid 10 µg | 32.3333 ± 0.701 |
| Glycopeptides | Vancomycin 5 µg | 14.3333 ± 1.402 |
| | Teicoplanin 30 µg | 16 ± 1.214 |
| Ansamycins | Rifampicin 5 µg | 25.3333 ± 0.701 |
| Amphenicols | Chloramphenicol 30 µg | 23.3333 ± 0.701 |

Table V. Testing of *MRSA* (internal code GR 0333), with various antibiotics combined with silver nanoparticles 10 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation).

| Antibiotic class | Antibiotic and silver nanoparticles | Zone of Inhibition (mm±SD) |
|------------------|--|----------------------------|
| Beta-lactamins | Penicillin 1 µg + AgNps 10 µg/mL | 6.3333 ± 0.701 |
| Cephalosporins | Cefoxitin 30 µg + AgNps 10 µg/mL | 13.3333 ± 0.701 |
| Macrolides | Erythromycin 15 µg + AgNps 10 µg/mL | 12.3333 ± 0.701 |
| Lincosamides | Clindamycin 2 µg + AgNps 10 µg/mL | 32.3333 ± 0.701 |
| Aminoglycosides | Gentamicin 10 µg + AgNps 10 µg/mL | 30.3333 ± 0.701 |
| | Tobramycin 10 µg + AgNps 10 µg/mL | 31.3333 ± 0.701 |
| | Amikacin 30 µg + AgNps 10 µg/mL | 31.3333 ± 0.701 |
| Fluoroquinolones | Ciprofloxacin 5 µg + AgNps 10 µg/mL | 34.3333 ± 0.701 |
| | Levofloxacin 5 µg + AgNps 10 µg/mL | 36.3333 ± 0.701 |
| | Norfloxacin 10 µg + AgNps 10 µg/mL | 31.3333 ± 0.701 |
| Tetracyclines | Tetracycline 30 µg + AgNps 10 µg/mL | 12.3333 ± 0.701 |
| Amphenicols | Chloramphenicol 30 µg + AgNps 10 µg/mL | 23.3333 ± 0.701 |
| Ansamycins | Rifampicin 5 µg + AgNps 10 µg/mL | 25.3333 ± 0.701 |
| Glycopeptides | Vancomycin 5 µg + AgNps 10 µg/mL | 15.3333 ± 0.701 |
| Oxazolidinones | Linezolid 10 µg + AgNps 10 µg/mL | 28.3333 ± 0.701 |
| Sulfonamides | Biseptol 25 µg | 30.3333 ± 0.701 |
| | Fusidic Acid 10 µg | 32.3333 ± 0.701 |

Silver nanoparticles with a concentration of 10 µg/mL developed an inhibition zone of 8.1429 ± 0.341 mm (Table VI). Antibiotics combined with 10 µg/mL silver nanoparticle solution did not develop significant areas of inhibition. Silver nanoparticles with a concentration of 100 µg/mL developed an inhibition zone of 16 mm, proving that increasing the concentration of the solution leads to a directly proportional increase in the area of inhibition, being effective as a bactericide.

Table VI. Testing of *MRSA* (internal code GR 0333), with silver nanoparticles 10 µg/mL and 100 µg/mL concentration and the obtained zone of inhibition (mm ± Standard Deviation).

| Silver nanoparticles | Zone of Inhibition (mm±SD) |
|----------------------|----------------------------|
| AgNps 10 µg/mL | 8.1429 ± 0.341 |
| AgNps 100 µg/mL | 16 |



Figure 14. MRSA testing to penicillin, 3rd-generation cephalosporins, silver nanoparticles and their combinations.

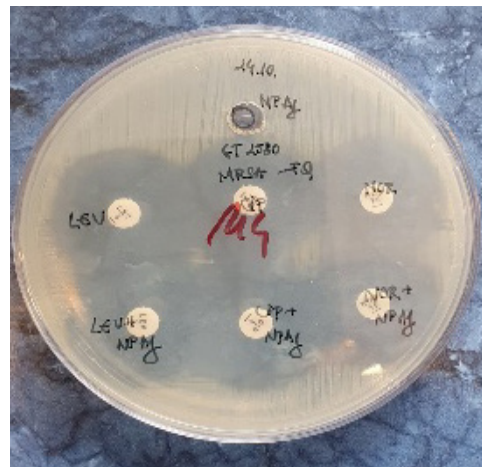


Figure 17. MRSA testing to fluoroquinolones, silver nanoparticles and their combinations.

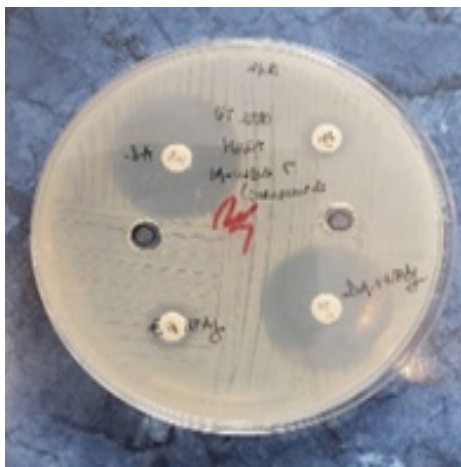


Figure 15. MRSA testing to macrolides, lincosamides, silver nanoparticles and their combinations.

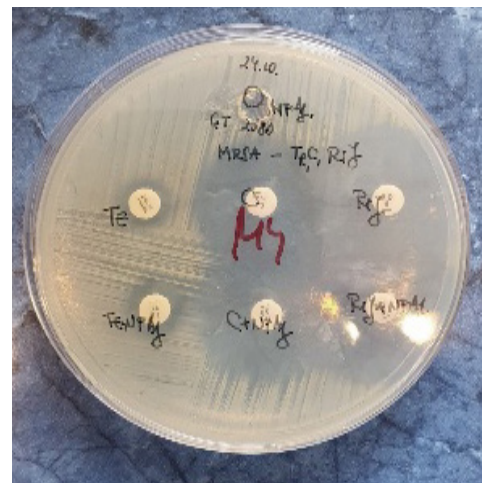


Figure 18. MRSA testing to other antibiotics (Rifampicin, Tetracycline and Chloramphenicol) silver nanoparticles and their combinations.



Figure 16. MRSA testing to aminoglycosides, silver nanoparticles and their combinations.

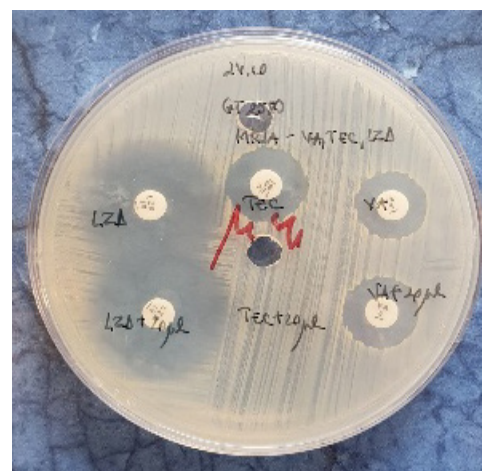


Figure 19. MRSA testing to glycopeptides, oxazolidinones, silver nanoparticles and their combinations.

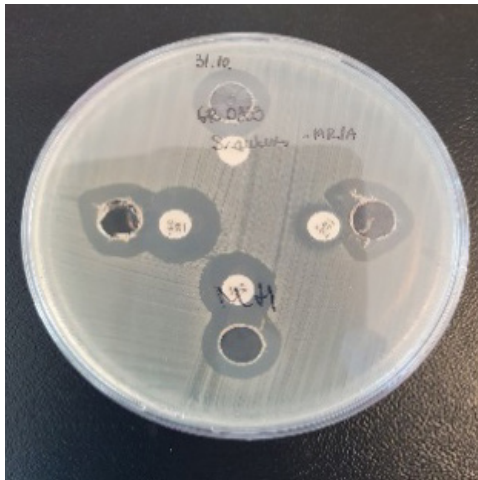


Figure 20. MRSA testing to penicillins, glycopeptides, second-generation cephalosporins and silver nanoparticles 100 µg/mL.

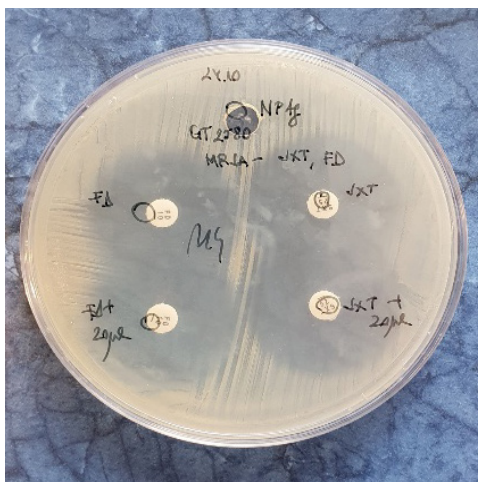


Figure 21. MRSA testing to Bisepitol, Fusidic acid and silver nanoparticles.

We tested *E coli* ATCC 25922 at several classes of antibiotics such as beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines, sulfonamides, nitrofurans and fosfomycins (Table VII).

When testing *E coli* ATCC 25922 bacterium at the betalactamins, we obtained areas of inhibition 18.3333 ± 0.701 mm for Ampicilin and 20.3333 ± 0.701 mm for Augumentine. When testing *E coli* ATCC 25922 bacterium at the fluoroquinolones antibiotic class (Figure 22), we obtained areas of inhibition above 26 mm for all 3 antibiotics tested, i.e. 26.3333 ± 0.701 for Ciprofloxacin, Levofloxacin and 27 mm for Norfloxacin. When testing *E coli* ATCC 25922 bacterium at the Tetracycline the inhibition zone was 19.3333 ± 0.701 mm (Figure 24). In the case of *E coli* ATCC 25922 testing for aminoglycosides (Figure 25), the highest value of the inhibition zone was obtained for Amikacin, 20 mm. In the case of *E coli* ATCC 25922 testing for Ampicillin we obtained the inhibition zone 18.3333 ± 0.701 mm (Figure 26). The highest zone of inhibition was found in *E coli* testing for Fosfomycin, 38 mm (Figure 27).

In the case of testing the bacterium with aminoglycosides combined with silver nanoparticles with a concentration of 100 µg / ml we obtained inhibition zones between 19 mm for Tobramycin and 20.3333 ± 0.701 mm for Gentamicin and Amikacin (Table VIII).

The highest area of inhibition, by 39 mm was found in *E coli* ATCC 25922 testing for Fosfomycin combined with silver nanoparticles with a concentration of 100 µg/ml (Figure 27).

We obtained an inhibition zone of 11 mm for silver nanoparticles with a concentration of 10 µg/mL and 12.5714 ± 0.88 mm for silver nanoparticles with a concentration of 100 µg/mL, proving the effectiveness of this more concentrated solution of silver nanoparticles as a bactericide (Table IX).

In the figure 23, we obtained synergism between silver nanoparticles and ciprofloxacin.

Table VII. Testing of *E coli* ATCC 25922 with various antibiotics and the obtained zone of inhibition (mm±Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm±SD) |
|------------------|-----------------------|----------------------------|
| Beta-lactamins | Ampicillin 10 µg | 18.3333 ± 0.701 |
| | Augumentin 30 µg | 20.3333 ± 0.701 |
| | Gentamicin 10 µg | 17.3333 ± 0.701 |
| Aminoglycosides | Tobramycin 10 µg | 18.3333 ± 0.701 |
| | Amikacin 30 µg | 19 |
| | Ciprofloxacin 5 µg | 26.3333 ± 0.701 |
| Fluoroquinolones | Levofloxacin 5 µg | 26.3333 ± 0.701 |
| | Norfloxacin 10 µg | 27 |
| | Tetracyclines | Tetracycline 30 µg |
| Sulfonamides | Bisepitol 25 µg | 27 |
| Nitrofurans | Nitrofurantoin 100 µg | 19 |
| Phosphonic Acid | Fosfomycin 200 µg | 38 |

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Table VIII. Testing of *E coli* ATCC 25922 with various antibiotics combined with silver nanoparticles 100 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation).

| Antibiotic class | Antibiotic and silver nanoparticles | Zone of Inhibition (mm±SD) |
|------------------|--|----------------------------|
| Aminoglycosides | Gentamicin 10 µg + AgNps 100 µg/mL | 20 |
| | Tobramycin 10 µg + AgNps 100 µg/mL | 19 |
| | Amikacin 30 µg + AgNps 100 µg/mL | 20.3333 ± 0.701 |
| Tetracyclines | Tetracycline 30 µg+ AgNps 100 µg/mL | 21 |
| Nitrofurans | Nitrofurantoin 100 µg+ AgNps 100 µg/mL | 21.3333 ± 0.701 |
| Phosphonic Acid | Fosfomycin 200 µg+ AgNps 100 µg/mL | 39 |

Table IX. Testing of *E coli* ATCC 25922 with silver nanoparticles 10 µg/mL and 100 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation.)

| Silver nanoparticles | Zone of Inhibition (mm±SD) |
|----------------------|----------------------------|
| AgNps 10 µg/mL | 11 |
| AgNps 100 µg/mL | 12.5714 ± 0.88 |

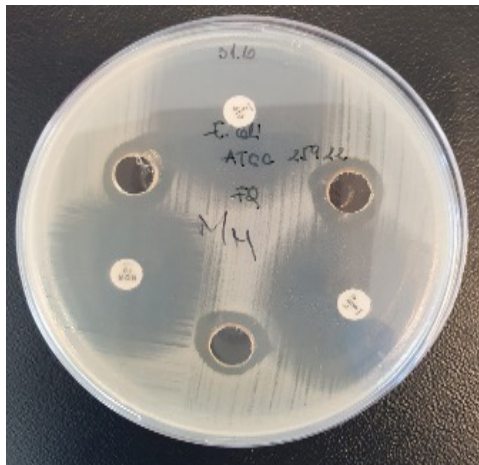


Figure 22. Testing of *E coli* to fluoroquinolones and silver nanoparticles.

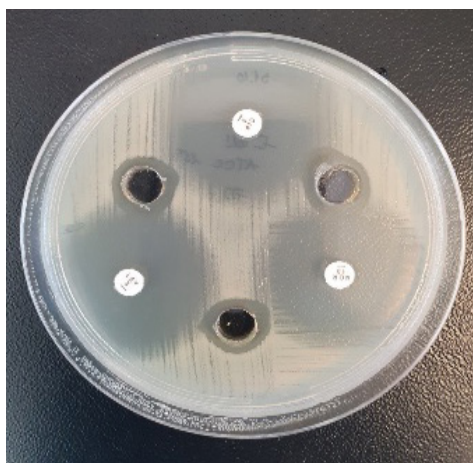


Figure 23. Testing *E coli* to fluoroquinolones and silver nanoparticles with the appearance of synergism.

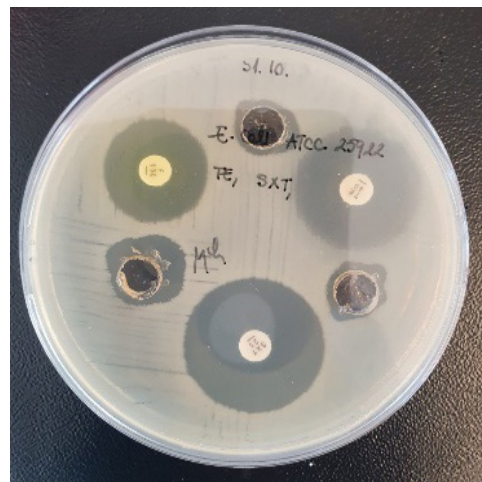


Figure 24. Testing *E coli* to other antibiotics (tetracyclines, sulfonamides and nitrofurans) and silver nanoparticles.

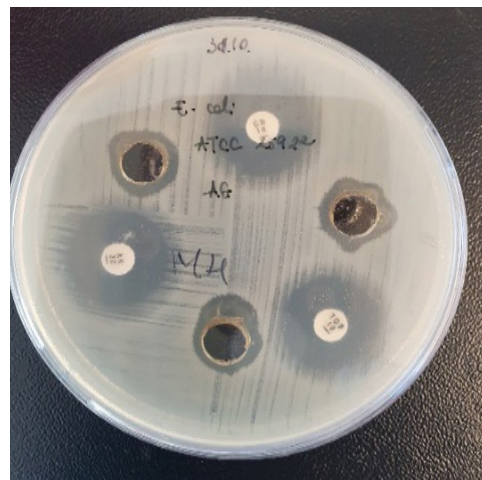


Figure 25. Testing *E coli* to aminoglycosides and silver nanoparticles.

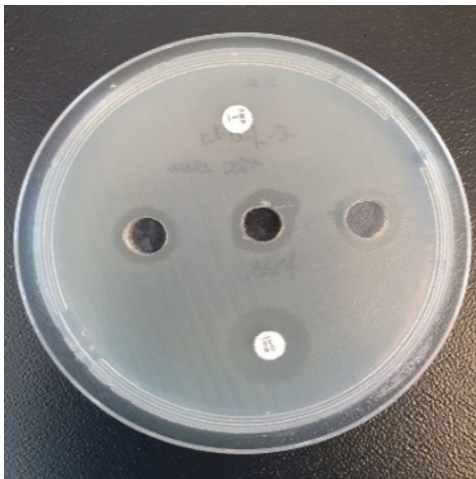


Figure 26. Testing *E. coli* to other antibiotics and silver nanoparticles.

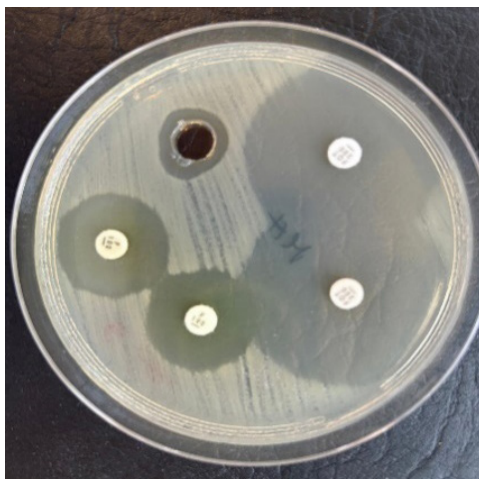


Figure 27. Testing *E. coli* to other antibiotics (nitrofurans and fosfomycins) and silver nanoparticles.

In the case of *K pneumoniae* (internal code GQ 8575) testing (Table X), we obtained lower inhibition zones when tested with carbapenems and cephalosporins of the fourth generation and higher when testing with silver nanoparticles (Figure 28), proving in this case that the antibacterial effect is more pronounced in the case of silver nanoparticles with a concentration of 100 $\mu\text{g/mL}$ (Table

XI). Antibiotics tested on this plaque have not proven effective as a bactericidal agent against this bacterium. On the culture medium plate, a slight synergistic effect is observed between the Cefepim disc (FEP) and the area of inhibition given by silver nanoparticles from well No 3.

Table XI. Testing of *K pneumoniae* (internal code GQ 8575) with silver nanoparticles 10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ concentration and the obtained zone of inhibition (mm \pm Standard Deviation).

| Silver nanoparticles | Zone of Inhibition (mm \pm SD) |
|----------------------------|----------------------------------|
| AgNps 100 $\mu\text{g/mL}$ | 12.3333 \pm 0.701 |



Figure 28. *K pneumoniae* (internal code GQ 8575) testing to carbapenems, fourth-generation cephalosporins and silver nanoparticles.

When testing *P aeruginosa* ATCC 27853 (Figure 29) on ticacillin 75 μg , penicillin class antibiotic, carboxypenicillin subclass, the inhibition zone was 15 mm and in Ceftazidime, third generation cephalosporins, the inhibition zone was 22.3333 \pm 0.701 mm (Table XII).

Table X. Testing of *K pneumoniae* (internal code GQ 8575) with various antibiotics and the obtained zone of inhibition (mm \pm Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm \pm SD) |
|------------------|----------------------------|----------------------------------|
| Carbapenems | Imipenem 10 μg | 9 |
| | Meropenem 10 μg | 6.3333 \pm 0.701 |
| Cephalosporins | Cefepime 30 μg | 6.3333 \pm 0.701 |

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Table XII. Testing *P aeruginosa* ATCC 27853 with various antibiotics and obtained zone of inhibition (mm ± Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm±SD) |
|------------------|-------------------|----------------------------|
| Beta-lactamins | Ticarcillin 75 µg | 15 |
| | Gentamicin 10 µg | 21.3333 ± 0.701 |
| Aminoglycosides | Tobramycin 10 µg | 21 |
| | Amikacin 30 µg | 25.3333 ± 0.701 |
| Cephalosporins | Ceftazidime 10 µg | 22.3333 ± 0.701 |

Table XIV. Testing *E faecalis* ATCC 29212 with various antibiotics and obtained zone of inhibition (mm±Standard Deviation).

| Antibiotic class | Antibiotic tested | Zone of Inhibition (mm±SD) |
|------------------|-------------------|----------------------------|
| Glycopeptides | Vancomycin 5 µg | 7 |
| Aminoglycosides | Gentamicin 30 µg | 18.3333 ± 0.701 |
| Beta-lactamins | Ampicillin 2 µg | 14.3333 ± 0.701 |

Table XV. Testing *E faecalis* ATCC 29212 with various antibiotics combined with silver nanoparticles 10 µg/mL and the obtained zone of inhibition (mm± Standard Deviation).

| Antibiotic class | Antibiotic and silver nanoparticles | Zone of Inhibition (mm±SD) |
|------------------|-------------------------------------|----------------------------|
| Glycopeptides | Vancomycin 5 µg + AgNps 10 µg/mL | 8.3333 ± 0.701 |
| Beta-lactamins | Ampicillin 2 µg + AgNps 10 µg/mL | 15.3333 ± 0.701 |
| Aminoglycosides | Gentamicin 30 µg + AgNps 10 µg/mL | 19 |

In the case of testing *P aeruginosa* ATCC 27853 for aminoglycosides (Figure 30) we obtained inhibition zone values above 21 mm for all 3 antibiotics tested, but the silver nanoparticles solution 10 µg/mL obtained an inhibition zone of only 8 mm proving that the low concentration of silver nanoparticles has a low bactericidal effect (Table XIII). When testing this bacterium with 100 µg/mL silver nanoparticles, the inhibition zone obtained was 16 ± 1.214 mm, proving that it is more effective than the first antibiotic tested. The inhibition zone has a slightly increased value compared to that of *E coli*, for silver nanoparticles with this concentration. Lowering the concentration of silver nanoparticle solution to 10 µg/ml decreases the bactericidal effect by half, as evidenced by the size of the inhibition zone of only 8 mm (Table XIII).

Table XIII. Testing *P aeruginosa* ATCC 27853 with silver nanoparticles 10 µg/mL and 100 µg/mL concentration and the obtained zone of inhibition (mm± Standard Deviation).

| Silver nanoparticles | Zone of Inhibition (mm±SD) |
|----------------------|----------------------------|
| AgNps 10 µg/mL | 8 |
| AgNps 100 µg/mL | 16 ± 1.214 |

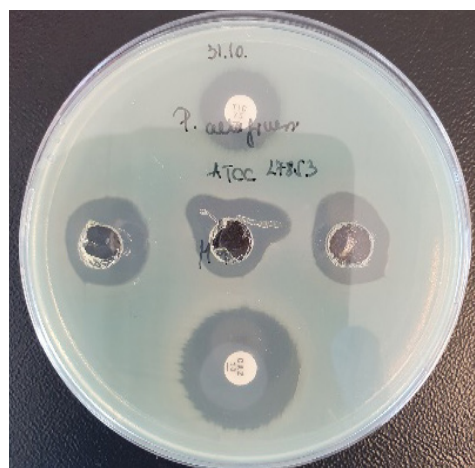


Figure 29. Testing *P aeruginosa* to ticarcillin and ceftazidim.

In the case of testing *E faecalis* ATCC 29212 for glycopeptides, aminoglycosides, penicillins (Table XIV), silver nanoparticles 10 µg/mL and combinations (Table XV), we obtained values of the inhibition zone between 7 and 19 mm (Figure 31).

Combining 10 µg/mL silver nanoparticles with tested antibiotics did not significantly increase the inhibition zone and we obtained similar values for Vancomycin and Gentamicin (Figure 31). In the case of Ampicillin combined with silver nanoparticles 10 µg/mL, the increase in the area of inhibition was only 1 mm (Table XV).

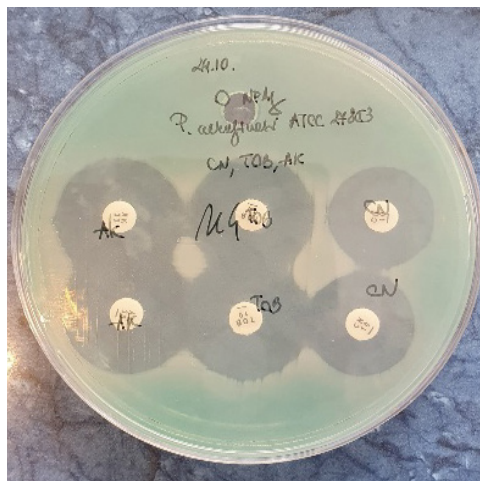


Figure 30. Testing *P. aeruginosa* to aminoglycosides.

The silver nanoparticles with a concentration of 10 $\mu\text{g/mL}$ achieved an inhibition zone of about 8 mm, similar to the area given by Vancomycin and half the value of that produced by Gentamicin, proving that small concentrations of silver nanoparticles do not exert a significant bactericidal effect (Table XVI).

Table XVI. Testing *E. faecalis* ATCC 29212 with silver nanoparticles 10 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ concentration and the obtained zone of inhibition (mm \pm Standard Deviation).

| Silver nanoparticles | Zone of Inhibition (mm \pm SD) |
|---------------------------|----------------------------------|
| AgNps 10 $\mu\text{g/mL}$ | 8.3333 \pm 0.701 |



Figure 31. Testing of *E. faecalis* ATCC 29212 to glycopeptides, aminoglycosides, penicillin, silver nanoparticles 10 $\mu\text{g/mL}$ and combinations.

Discussion

As described, the main cause of nosocomial infections is the emergence of bacterial strains resistant to antibiotics [3]. Infections caused by multidrug-resistant bacteria require complex antibiotic therapy, in increased doses and for a longer time. This increases their toxicity to the human body, worsening the patient's condition, even causing death.

The ability of certain bacteria to form biofilm further increases their resistance to antibiotics, often characterized by a slower wound healing rate, and a prolonged hospitalization period [22].

Given their unique and special properties, silver nanoparticles have demonstrated clear antibacterial effects especially drug multiresistant ones, thus making them a viable treatment alternative [2,4,19,31-33].

In the article published in 2022, Asif et al. show that the antibacterial effect of silver nanoparticles is conditioned by the concentration of the solution used. When testing *E. coli* bacteria with silver nanoparticles of different concentrations, the areas of inhibition were different [32]. We have obtained similar results to Asif et al. in terms of demonstrating that an increase in concentration of silver nanoparticles solutions produce a larger inhibition zone. The solutions we produced range from 10 to 100 $\mu\text{g/mL}$ and the resulting zones of inhibition are between 11 and 12.5714 \pm 0.88 mm. Asif et al. used solutions ranging from 25 to 100 $\mu\text{g/mL}$ and the resulting zones of inhibition are between 15 to 19 mm, thus enforcing the statement that a higher concentration of silver nanoparticles has a superior antibacterial effect.

Hussain et al., in their 2023 article, showed that the size of the silver nanoparticle inhibition zones for several types of bacteria tested is directly proportional to the concentration of nanoparticles used for testing (Table XVII) [34]. This hypothesis was also demonstrated in this study, when the concentration of 10 $\mu\text{g/mL}$ of silver nanoparticle solution was less effective compared to the concentration of 100 $\mu\text{g/mL}$.

We have compared our results to a second study [34] and we can deduce that for the solution of 100 $\mu\text{g/mL}$ concentration, we obtained similar base result of around 14 mm inhibition zone for *S. aureus*. When combining the nanoparticle solution with various antibiotics, we have obtained a much higher inhibition zone up to 32 mm. This proves the combination of various antibiotics with silver nanoparticles increases the antibacterial activity at least 2-fold.

The inhibition zone for *E. coli* in our study was approximately 13 mm, while Husain et al. obtained a higher inhibition zone. This difference can be explained by the size of the silver nanoparticles, the synthesis method, and the exposure time to the studied bacteria. However, when we combined the antibiotics with the silver nanoparticle solution, our inhibition zones increased to 39 mm, triple in size.

Table XVII. Zone of inhibition of AgNps against different bacteria at different concentrations (mm±SD).

| Bacterial strains | Zone of inhibition of AgNps against different bacteria at different concentrations (mm±SD) | | |
|-----------------------------|--|-------------|-------------|
| | 100 µg/mL | 75 µg/mL | 50 µg/mL |
| Genus <i>Staphylococcus</i> | 12 ± 0.68 | 10 ± 0.20 | 0.8 ± 0.15 |
| <i>E coli</i> | 19.7 ± 0.76 | 18.2 ± 0.66 | 15.4 ± 1.15 |

Other studies show that the antimicrobial effect of silver nanoparticles is conditioned by several factors, of which their size and shape are essential. It has been shown that the smaller and more spherical the silver nanoparticles, the more pronounced their antibacterial effect is [1,14,35]. Combining them with antibiotics increases the bactericidal effect in most cases, as shown by numerous studies in the literature [5,14,20,23,34-37].

In this study, Vancomycin was used to test antibacterial activity for *S aureus*, MRSA and *E faecalis*, being effective in the first two bacteria and less effective for *E faecalis*. Vancomycin combined with silver nanoparticles, developed an increased zone of inhibition in all bacteria studied, demonstrating the superior bactericidal effect of the combination.

The same results were recorded in another article published in 2022, by Salah et al., where increased values of the inhibition zone were obtained for the combination of Vancomycin with silver nanoparticles [28].

Kaur et al. in the study published in 2019, showed that Vancomycin is not effective against gram-negative bacteria, respectively *E coli*, but the combination of Vancomycin with silver nanoparticles is effective, although none of the 2 bacteria reacted significantly to the administration of silver nanoparticles. The combination of amikacin with administered silver nanoparticles resulted in a higher area of inhibition for *E coli* compared to that for *S aureus* (Table XVIII) [36].

Table XVIII. Zone of inhibition(mm) for *S aureus*.

| Tested substance and concentration used | Zone of inhibition(mm) for <i>Staphylococcus aureus</i> |
|---|---|
| AgNps 60µg/mL | 0 |
| Vancomicina 0.5 mM | 7 |
| Vancomicina+ AgNps 0.5 mM | 11 |
| Amikacina 0.3 mM | 4 |
| Amikacina+ AgNps 0.3 mM | 10 |

Other articles published after 2019 show that the combination of silver nanoparticles with antibiotic has a higher bactericidal effect compared to that of only the antibiotic [15,16,19,25,32,33,37-39]. In this paper, superior bactericidal effect was demonstrated for combinations of nanoparticles and antibiotics in all cases.

Conclusion

The combination of silver nanoparticles with different classes of antibiotics has been demonstrated to be more effective, developing a higher zone of inhibition when compared to just using antibiotics. The efficiency is influenced by the concentration of the silver nanoparticle solution.

Infections caused by multidrug-resistant bacteria are associated with increased morbidity and mortality and high treatment costs. The use of metallic nanoparticles, especially silver ones, as antimicrobial agents with definite bactericidal activity has led medical specialists to consider this new treatment which may change antibacterial therapy.

Studies of in vitro combinations between silver nanoparticles and different classes of antibiotics represent a highly efficient and effective new antibacterial treatment against multidrug-resistant bacteria. To avoid the problem of antimicrobial resistance associated with conventional antibiotics, it is necessary to understand the adaptive mechanisms of bacteria under the action of metal nanoparticles, which could be exploited in future studies.

Considering their special properties, silver nanoparticles possess all the qualities to become the drugs of the future, after cytotoxicity is resolved and stability and biocompatibility are improved.

More in vivo studies are needed to achieve satisfactory results in antibacterial therapy for silver nanoparticles to become the new class of drugs capable of fighting multidrug-resistant bacteria when classical antibiotics do not provide the expected results.

Acknowledgement

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