Antibacterial Effect of Silver Nanoparticles on Klebsiella spp

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Research Article

Abstract

Silver nanoparticles (AgNP) can be incorporated into medical devices, such as tissues, to circumvent bacterial resistance such as Klebsiella spp, which can lead to skin and mucosal infections. Thus, the aim of the present study was to synthesize silver nanoparticles for later incorporation into cotton fabrics and in vitro tests against Klebsiella spp. The AgNP colloidal solution was synthesized (AgNO3 - 0.1 mM, 100 mM trisodium citrate, polyvinylpyrrolidone - 0.24 g, H2OH2) and then impregnated into the cotton fabric pretreated with poly diallyl dimethylammonium chloride (PDDA) of 100/500 tissue, shaken for 30 minutes). The material produced was analyzed by the FTIR; DLS and reflectance spectroscopy. The tests of the antimicrobial activities were by the microdilution technique against Klebsiella spp, in tubes containing Brain Heart Infusion (BHI), with the solution of silver (1); Tissue containing AgNP - 4 mm (2); Negative control (3) and positive control - ceftriaxone (4). Regarding MIC, the inhibitory activity occurred of the dilutions between 1/2 and 1/16. The AgNP particles had an average size of 24.75 nm. As synthesized AgNPs demonstrate the excellent antimicrobial activity against Klebsiella spp, with special emphasis on applications in nanotechnology and nanomedicine, targeting multiresistant antibiotic bacteria.

Keywords: Nanoparticles; Silver; Antibacterial; Activity.

1. Introduction

The genus Klebsiella comprises a group of Gram-negative bacilli bacteria from the Enterobacteriaceae family. These microorganisms are considered opportunistic since they are associated with hospitalized and immunocompromised patients, which can lead to serious

infections of the respiratory tract, urinary tract, mucous membranes, and skin (burns and wounds).[1,2]
This bacterium can survive on the skin and dry environments for a long time, being a reason for growing concern, as there have been increasing cases of multi-resistance to antibiotics, especially in skin infections, since some species are producing Betalactamases causing the potential cause of morbidity and mortality among hospitalized patients.[1,3,4]
This way, the development of tissues and solutions for application in this area becomes relevant, among which the metallic ions of nanoparticles, especially silver nanoparticulate (AgNp) in silver, are effective materials developed in medical textiles.[5, 6]
The use of silver nanoparticles is justified, since the simple impregnation of silver nitrate in fabrics is not adequate, because contact with light and air, the material tends to be dyed dark brown. In addition, the smaller the silver particles, the greater the antimicrobial effect.[5,6]
The antimicrobial effect of silver nanoparticles is due to the damage they cause to the bacterial membrane (membrane bonding), alteration of bacterial DNA, and compromise of bacterial protein synthesis.[6, 7, 8] The present study aimed to analyze the antimicrobial properties of silver and tissue nanoparticles solution impregnated with that material against Klebsiella spp.

2. Methodology

2.1 Preparation of the silver nanoparticle solution

Initially, aqueous solutions of $AgNO_3$ (0.1 mM, 200 mL), tri-sodium citrate (100 mM, 3.6 mL), PVP (0.24 g) and H_2O_2 (30 wt. %, 0.48 mL) were mixed vigorously at room temperature in air. Then, 0.85 mL of the $NaBH_4$ solution (100 mM) was rapidly added into the mixture to initiate the reduction under room dark. After approximately 30 min, Rubra colloidal solution was obtained by Ag nanoplates formed. The colloidal nanoparticle solution was then stored in a refrigerator for future characterization.[9, 10]

2.2 Preparation of the cotton cloth and impregnation of the silver colloidal solution

The industrialized cotton fabrics were initially washed with deionized water and then submerged in an aqueous solution (2%) of poly-diallyl dimethylammonium chloride (PDDA) for 12 hours. At the end of that period, the tissue was washed with deionized water and dried at room temperature. Subsequently, the PDDA-treated tissues were immersed in the solution of silver nanoparticles in the solution-tissue ratio of 100/500 and shaken for 30 minutes. In the end, it was washed with deionized water and dried at room temperature.[9]

2.3 Preparation of the bacterial suspension

Klebsiella spp used in this study was obtained from the multidisciplinary laboratory of the Traíri Health Sciences School, Rio Grande do Norte / Brazil, and cultivated in BHI (Brain Heart Infusion) medium at 37 ° C / 24 h. This bacterium was selected due to its initial isolation in a university hospital, showing resistance in this environment. Thus, further studies are required for the development of tissues that can help to circumvent the resistance of this strain. Bacteria were then resuspended in 5 mL sterile saline (0.89% NaCl) until reaching the turbidity equivalent to the Mac Farland 0.5 scale (1.5 x 108 bacteria / mL).[11, 12]

2.4 Determination of antibacterial activity by the microdilution method

In this step, sterile tubes containing 1.5 mL of BHI broth were used as a positive control (1), negative control (2), silver solution (3), and silver tissue (4). In each tube 50 µL of the bacterial suspension of Klebsiella spp. Positive control (ceftriaxone), negative control (1.5 mL of deionized water), silver solution (1.5 mL of silver nanoparticle solution), and silver tissue (4 mm of

impregnated tissue) were added to the tubes with silver nanoparticle). In the end, they were taken to the bacteriological oven and kept for 24h.[11, 12]

2.5 Determination of Minimum Inhibitory Concentration (MIC)
Initially sterile tubes containing 1.5 mL of BHI broth were numbered for analysis of the MIC of the silver nanoparticle solution, which was serially diluted (1 / 2.1 / 4.1 / 8.1 / 16, 1/32, 1/64). In each tube was added 50 µL of the bacterial suspension of *Klebsiella spp*. Then, 1.5 mL of the diluted solution was added to each specific tube, followed by homogenization and incubation at 37°C / 24h in a bacteriological oven. The MIC was the lowest concentration of the silver nanoparticle solution where there was no visible bacterial growth.[11]

2.6 Characterization of the silver nanoparticle (AgNP)
Dynamic Light Scattering Analysis (DLS): Dynamic light scattering (DLS) analysis was performed to measure the main particle size of the AgNPs in a colloidal solution. The sample was initially sonicated for 5 min to obtain a uniform dispersion of the particles followed by the four-scan analysis - Brookhaven Nanobrook apparatus - model: 90plus PALS.[13]

Absorption analysis by UV - visible spectroscopy: Characterization of the AgNP-impregnated tissue was analyzed using UV - visible spectroscopy. The silver ions reduce into nanoparticles was monitored by measuring the absorbance of the reaction buffer in the 400-700 nm range - Shimadzu Spectrophotometer - model: UV-2600.

Fourier Transform Infrared Spectroscopy (ATR-FTIR): The synthesized particle and the tissues used in the study were characterized by ATR-FTIR spectrophotometry, through a scanning area between 3750-500 cm $^{-1}$, with 128 repetition scans and resolution adjusted in 4 cm $^{-1}$ - BRUKER - model: FT -IR VERTEX 70.

3. Results and discussions

3.1 Synthesis and characterization of AgNP
The synthesis of the red silver nanoparticle (AgNP) was performed successfully, as shown in the figure, at the end of the process, to red silver nanoparticles, with a color change in the solution (Figure 01 - A). From this colored solution, the treatment of the cotton fabric was carried out, obtaining the result, presented in Figure 02 - B.

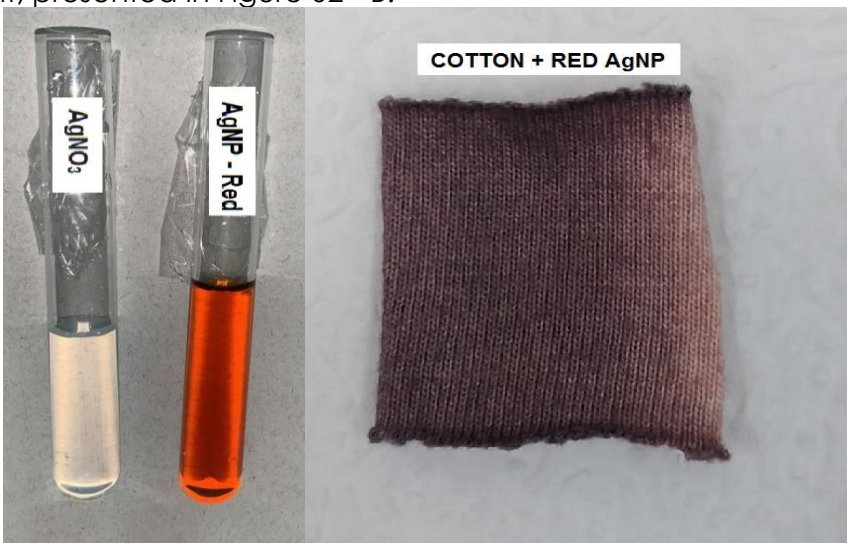


Figure 01: A - AgNP red solution synthesized; B - Cotton fabric impregnated with red AgNP.

The present synthesis was achieved by the reduction of silver ions (Ag^{+2}) in an aqueous solution, which is achieved by the use of sodium citrate and/or sodium borohydride, favoring the formation of free metallic silver (Ag^{o}), forming at the end the AgNPs, that was determined by the change in coloration of the material to the color red. Complementarily, the addition of PVP contributes to stabilizing the AgNPs, preventing their agglomeration.[9, 10, 13]

The first characterization was performed with the silver nanoparticle colloidal solution, which was analyzed through the Dynamic Light Scattering Analysis (DLS), which is based on the diffraction of the laser in a sample in order to obtain the particle size in solution (Table 01).

Table 1: Determination of silver nanoparticle size (DLS)

Polydispersity	Diameter (nm)
0,501	18,99
0,470	33.43
0,288	28,13
0,445	18,46
Size (nm)	**24,75**

The geometry and size of the AgNPs with the incidental light generate the polydispersity, which allows the analysis of the average particle size in a colloidal solution, as described by Aragão et al. [14]. As shown in Table 1, the colloidal solution obtained presents small size Ag nanoparticles, which contributes to the bactericidal activity of AgNPs, since it facilitates integration with the membrane and penetration into the bacterial structure, which can damage DNA and protein synthesis.[12, 13, 15]

The cotton cloth impregnated and not impregnated with AgNP was analyzed by diffusion reflectance spectrophotometry in order to verify the color content after the adsorption process. It is observed that the tissue with AgNPs rubber presents peaks of greater intensity between 450-500 nm (figure 02).

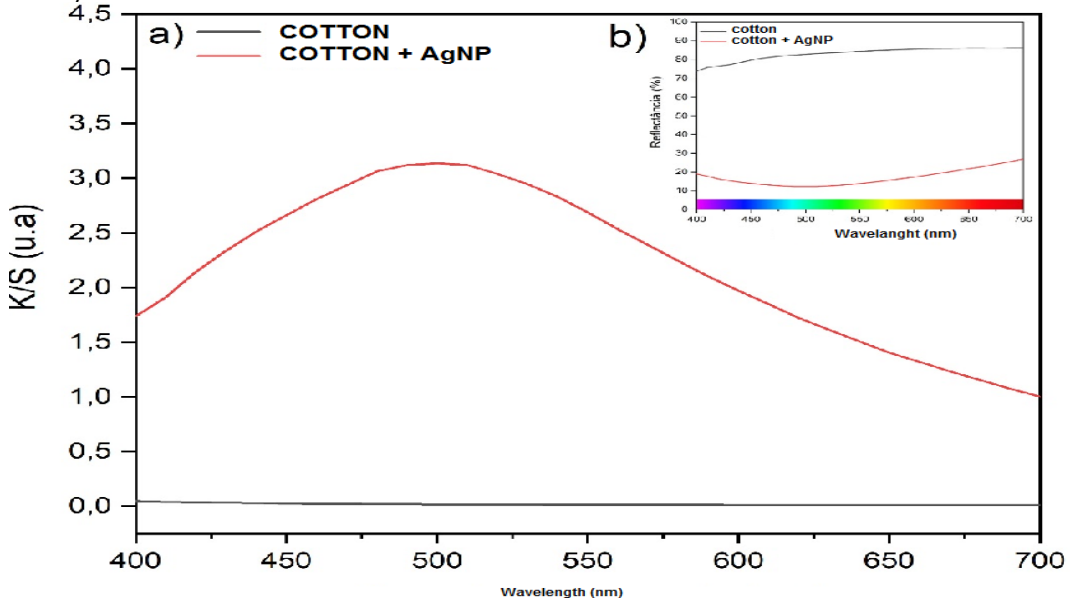
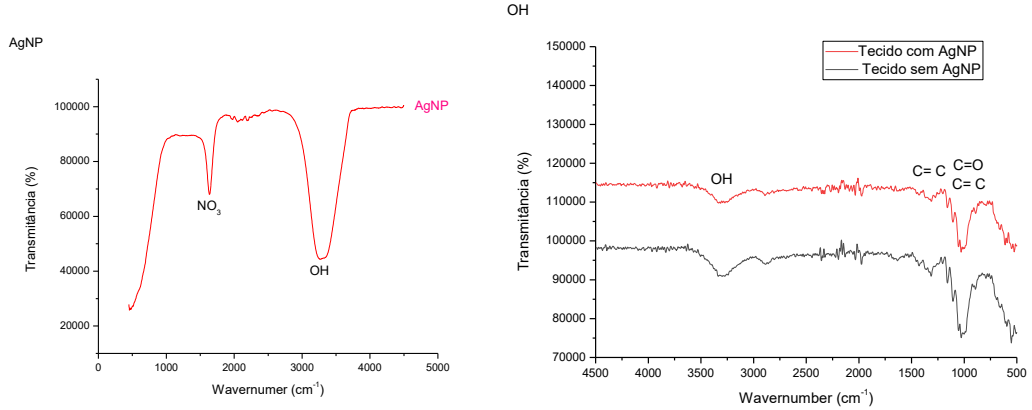


Figure 2: A) Coloristic strength (K / S) and b)% Diffuse reflectance of cotton fabrics (black line) and red silver nanoparticles (red line)

As shown in figure 02-A, it is possible to observe that the cotton fabric not treated with AgNPs, does not show absorptivity because the incidental light is mostly reflected in the whole spectrum range visible by the in natural cellulosic fiber. On the other hand, tissue impregnated with AgNP rubber shows a characteristic absorbance range of greater intensity, due to the excitation mode of its surface plasmon between 450-500 nm, associated with the optical property of the silver nanoparticles.[15, 16]

Figure 3 shows the FTIR spectrum of AgNP (A) with peaks of greater intensity between 1500-1610 cm^{-1} and 3,200-3,100 cm^{-1}. However, the spectra of the tissues used in study (B) show higher peaks between 3300-3400 cm^{-1}, 1200-1350 cm^{-1}, and 2000-2 2100 cm^{-1}.



A **B**

Figure 03: FTIR spectrum: A - Ag nanoparticle; B - Inherent tissue and tissue with AgNP

The bands observed in the AgNP spectra refer to NO_3 and OH vibration peaks that are typical of the nanoparticle synthesis.[17, 18] The spectrum of tissues exhibit peaks of OH, C = C, and C = O, typical material of organic material such as cotton derivatives. It was possible to observe that the peaks of the treated tissue, especially OH (3,300 - 3,400 cm^{-1}) reduced in intensity, demonstrating that there was an adsorption of the AgNPs in the tissue.

Therefore, it is possible to affirm that the treatment of the tissue with the nanoparticles solution did not affect the molecular structure of the tissue, since the peaks are characteristic in the two spectra, only occurring a reduction in the intensity of the peaks in the treated tissue, resulting from the adsorption of silver nanoparticles on the surface of treated tissue.[17, 18, 19]

3.2 Microbiological tests

Initially, a qualitative test was performed on the *in-vitro* inhibition capacity by the microdilution technique against *Klebsiella* spp by the AgNP rubber solution (Figure 01).

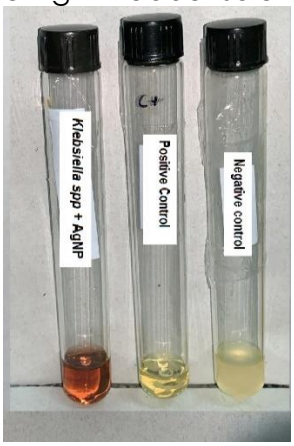


Figure 04: Analysis of antimicrobial activity by AgNP

As observed, the tube containing AgNP remained translucid, being the red color resulting from the solution of silver nanoparticle used, similar, in the absence of turbidity, to that observed in the positive control (ceftriaxone). On the other hand, the tube containing the negative control (deionized water) was cloudy, demonstrating that bacterial growth occurred. This activity is similar to that observed in other studies, being justified by AgNP's actions on *Klebsiella spp*, such as inhibition of metabolism and production of bacterial biofilms, in addition to structural damage.[20, 21]

The determination of the minimum inhibitory concentration was performed according to Silva et al. [11] and Suleiman et al. [12] by different dilutions (1 / 2.1 / 4.1 / 8.1 / 16, 1/32, 1/64). It was possible to observe bacterial growth inhibition effectiveness at dilutions between 1/2 and 1/16, as shown in figure 05.

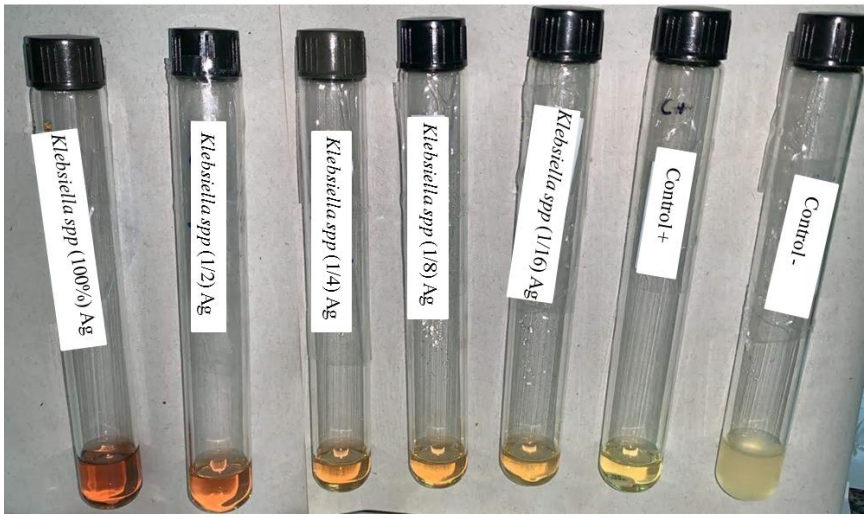


Figure 05: Analysis of the minimal inhibitory concentration of AgNP versus *Klebsiella spp.*

The bacterial sensitivity of silver nanoparticles is demonstrated in various concentrations, and they may have varied MIC according to the size, charge, and shape of the AgNP particle. In this study, the MIC was observed in 1/16, being within the study ranges observed by Prema et al [23] with MIC = 40 µg / ml.

This activity is justified because the AgNPs bind to the surface of the cell membrane and affect the structural permeability. In addition, they may enter the bacterial periplasm, promoting DNA damage and affecting bacterial metabolism.[22]

Finally, a qualitative test based on microdilution, adapted from Silva et al. [11] and Suleiman et al. [12], with the colloidal solution and the tissue impregnated with AgNPs, as shown in figure 06.

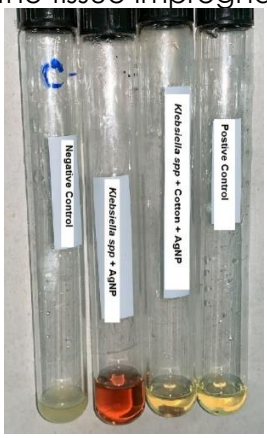


Figure 6: Analysis of the antimicrobial activity of the AgNP and tissue (cotton) + AgNP solution against *Klebsiella spp*.

As observed in the tubes containing the red AgNP colloidal solution, the AgNP treated tissue and the positive control (antibiotic) remained translucent in relation to the negative control, which contained untreated tissue, becoming cloudy due to *Klebsiella spp*. Thus, both AgNP in solution and impregnated in tissue were shown to inhibit bacterial proliferation in the analyzed period, similar to that observed in Balakumaran et al. [24] and Lorenz et al. [25].

The use of nanoparticles in the tissues generates a greater functionality, demonstrating its capacity of antimicrobial activity, is an important one for the treatment of hospital tissues, contributing to the inhibition of bacteria resistance to antibiotics, like *Klebsiella spp*, being able to mitigate risks of hospital infections.[20, 21, 26]

4. Conclusion

Based on the data presented, it is concluded that the silver nanoparticles presented average sizes of 24.75 nm, being possible the integration of them in the cotton fabric. The CIM results demonstrated that the synthesized colloidal dispersions show an antibacterial effect, at dilutions between 1/2 and 1/16 versus *Klebsiella spp*. Likewise, qualitative testing of tissue impregnated with AgNP demonstrated the ability to inhibit bacterial growth. In this context, new studies on tissue activity impregnated with colloidal nanosilver should be extended, so that they can provide an emerging strategy to combat disease-resistant pathogens in the future.

Conflict of Interest: The authors declare no conflict of interest.

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