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THE ACTION OF SILVER NANOPARTICLES ON BACTERIAL STRAINS

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Abstract: This study approaches an issue of great interest in bio-nanotechnology and nanomedicine, for example: the possibility of usage for various concentrations of colloidal silver to reduce or to neutralize the activity of certain bacteria inside organisms. The study takes into consideration reference strains such as: *Escherichia coli /ATCC 10536, Klebsiella pneumoniae/ ATCC 700603, Pseudomonas aeruginosa /ATCC 25668, Salmonella typhi/ ATCC 14028, Staphylococcus aureus/ ATCC 6538, Streptococcus pyogenes/ ATCC 19615* and nosocomial strains isolated from hospital units such as: *Escherichia coli/CCM 2371, Klebsiella pneumoniae/CCM 1986, Pseudomonas aeruginosa/CCM 8912, Salmonella typhi/CCM 381, Staphylococcus aureus/CCM 2877, Streptococcus pyogenes/CCM 8112*. The determinations were made by using the diffusion method (adapted after the Kirby-Bauer test) in the presence of colloidal silver. The results show that, in the presence of silver, the reference strains show an increased sensitivity than the nosocomial strains; the most relevant bactericide effects were observed by using a 20ppm colloidal solution.

Keywords: *antibacterial action, AgNPs, bacteria, diffusion*

INTRODUCTION

Nowadays, studies focus more and more on finding alternative methods to neutralize or reduce the activity of certain bacteria that may affect human health. Thus, studies have been conducted on plants and plant extracts, on essential oils and on silver or colloidal silver compounds.

In order to replace or to increase the potency of several antibiotics, studies have been focused on plant extracts that posses' antimicrobial properties [1 – 3]. Thus, Koshy et al. (2006) studied a series of anti-bacterial plants from Malaysia [4], Ali-Shtayeh et al. (2008) plants from Palestine [5] and Ljubuncic et al. (2006) studied *Teucrium polium* L. and its antioxidant action [6]. The antimicrobial activity of essential oils extracted from different plants analyzed on bacteria from the *Escherichia* genus, *Escherichia coli* species, were presented by Sandru (2015), and the most significant results were observed regarding the *Cinnamomum aromaticum* Nees*, Origanum vulgare* L. and *Abies alba* Mill essential oils. Moderate antimicrobial activity was determined for essential oils extracted from: *Teucrium marum* L.*, Thymus vulgaris* L.*, Hippophae rhamnoides* L.*, Lavandula angustifolia* Mill.*, Coriandrum sativum L.,* and the lowest antimicrobial activity was observed for essential oils extracted from: *Pinus sylvestris L., Salviae officinalis* L.*, Zingiber officinale* Roscoe. and *Anethum graveolens* L. [7]. Researchers have shown that many essential oils and plants have antifungal activity, not just antibacterial activity [1, 8]. Silver has demonstrated several antibacterial qualities regarding different bacterial species, even those with increased resistance to antibiotics or chemicals. Therefore, colloidal silver has prevailed as treatment in the cases where medication failed. Nanomedicine, including bio-nanotechnologies uses these nanoparticles as both therapeutic tools, and in various treatments on contaminated waters, in preventing the formation of bacteria colonies on catheters, since the activity of noble metals has successfully been tested. So, metal nanoparticles have different applications, such as electronic, catalysis and photonics [9 – 11]. Silver nanoparticles (AgNPs) can successfully replace a series of antibiotics, especially as they act against a broad spectrum of various bacterial strains, with no side effects [12]. Hospital infections are increasing because of bacterial (nosocomial) pathogens against which current antibiotic therapies are not efficient, as antimicrobial resistance is a major threat to human health [13]. The improper use of antibiotics has led to these forms of bacterial resistance; the result is a higher mortality caused by infection with these germs [14 – 16]. Although new drugs are always launched on the market, microorganisms become more and more resistant; the first step in fighting this is to reduce the use of antibiotics, but at the same time to optimize the pharmacokinetics and pharmacodynamics of the new medicines, with a view to reduce side effects and the risk of developing resistance [17 – 19].

Singh et al. 2014 studied the antibacterial activity of silver nanoparticles synthetized from *Tinospora cordifolia* Miers. on nosocomial *Pseudomonas aeruginosa* bacteria strains isolated from hospitals that treated patients with burn injuries. The synthetized silver nanoparticles were characterized using ultraviolet–visible spectroscopy, dispersive spectroscopy and infrared spectroscopy. Transmission electron microscopy and X-ray diffraction revealed that the silver nanoparticles' size is between 9 ± 36 nm, respectively 12.49 nm. The antibacterial activity of silver nanoparticles obtained from *Tinospora cordifolia* Miers. against nosocomial strains was determined through the

diffusion test, and inhibition intervals were found to be between 10 ± 0.58 and 21 ± 0.25 mm [20].

The synthesis of silver nanoparticles using *Sesamum indicum* L. extract and the antibacterial potential against the *Klebsiella pneumoniae* strain isolated from urinary tract infections was presented by Shirmohammadi et al. in 2014. The results obtained showed that there is a simple approach to ecologically synthetize silver nanoparticles using *Sesamum indicum* L. seed water extracts. The method is based on the quick change in plant extract color under the action of silver ions from a solution of *Sesamum indicum* L. seed water extracts. Benign natural AgNPs were further confirmed through UV-Vis spectroscopy; AgNPs biosynthesized from bamboo leaves also show intense antimicrobial activity, especially on *Klebsiella pneumoniae* strains [21].

Nanotechnology is a modern, innovative approach, which can be used to issue new formulations based on antimicrobial metallic nanoparticles. In 2011, Ansari et al. analyzed the microbial growth curve, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of silver nanoparticles (Ag-NPs) against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* sensitive to methicillin (MSSA) and against *Staphylococcus aureus* resistant to methicillin (SAMR). The results showed that the lowest MIC and MBC of Ag-NP were 12.5 $\mu g \cdot mL^{-1}$, respectively 25 $\mu g \cdot mL^{-1}$. The results obtained suggested that Ag-NP have an excellent bacteriostatic and bactericidal effect compared to all clinically isolated substances tested, regardless of their drug resistance mechanisms [22].

MATERIALS AND METHODS

To perform the study, reference strains and nosocomial strains of bacteria were used. The reference strains were represented by: *Escherichia coli* /ATCC 10536, *Klebsiella pneumoniae*/ ATCC 700603, *Pseudomonas aeruginosa* /ATCC 25668, *Salmonella typhi*/ ATCC 14028, *Staphylococcus aureus*/ ATCC 6538 and *Streptococcus pyogenes*/ ATCC 19615 and the nosocomial strains, by: *Escherichia coli/CCM 2371, Klebsiella pneumoniae/CCM 1986, Pseudomonas aeruginosa/CCM 8912, Salmonella typhi/CCM 381, Staphylococcus aureus/*CCM 2877 and *Streptococcus pyogenes/CCM 8112.*

The nosocomial strains were isolated for scientific purposes in hospitals, from anonymous patients. To determine the antimicrobial activity of silver nanoparticles, the diffusion method was used. The solutions used in this study were represented by colloidal silver solutions that had the following concentrations: 5 ppm, 10 ppm, 15 ppm and 20 ppm and a 0.1 N $AgNO_3$ solution.

The principle underlying the method consists of placing the disks holding colloidal silver on the surface of a solid medium inoculated with a bacterial culture. The active antimicrobial substance will diffuse in the medium, presenting a constant decrease of the concentration gradient, from around the micro tablet to the edge.

After a certain incubation period, two distinct areas will become visible: one in which the bacterial growth is inhibited by concentrations of antimicrobial substance, and a growth area where the silver concentration is too low to inhibit the growth.

The bigger the diameter of the inhibition zone, the more sensitive the germ is. The diameter of this zone and the minimum inhibitory concentration are inversely proportional.

The bacterial concentration used for this study was 10^6 CFU·mL^{-1}. The growth media used for the development of the bacteria were specific to each strain, as follows: AABTL (RO/IVD-093-167-37) for *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and blood agar (Scharlau gmbh, Scharlab/064-PA0059) for *Staphylococcus aureus, Streptococcus pyogenes.* The diameter of the sterile paper filter disks was 6 mm, and the silver solution deposited on them was determined at 100 µL·disk^{-1}. Incubation was established at 37 ^{0}C for 24 hours.

RESULTS AND DISCUSSIONS

Following the measurements, we determined that silver nanoparticles have different antibacterial actions - the values obtained can be consulted in Table 1.

Table 1. *Inhibition value of reference and nosocomial strains*

Bacterial strain	Inhibition value [mm]				
	Colloidal silver concentration				0.1N $AgNO_3$ solution
	5 ppm	10 ppm	15 ppm	20 ppm	
Escherichia coli ATCC 10536	6.2±0.02	6.5±0.08	6.6±0.01	8.8±0.04	7.4±0.1
Escherichia coli CCM 2371	6.0±0.01	6.0±0.01	6.1±0.04	6.2±0.01	6.4±0.01
Klebsiella pneumoniae ATCC 700603	6.7±0.03	7.2±0.07	7.8±0.02	8.2±0.08	8.1±0.09
Klebsiella pneumoniae CCM 1986	6.0±0.00	6.0±0.00	6.0±0.01	6.2±0.01	6.2±0.01
Pseudomonas aeruginosa ATCC 25668	6.8±0.01	6.8±0.07	6.9±0.02	7.9±0.01	7.1±0.04
Pseudomonas aeruginosa CCM 8912	6.0±0.04	6.0±0.01	6.2±0.03	6.7±0.05	6.8±0.08
Salmonella typhi ATCC 14028	7.0±0.02	7.0±0.05	7.2±0.01	8.5±0.04	8.2±0.01
Salmonella typhi CCM 8912	6.0±0.01	6.0±0.04	6.2±0.05	7.5±0.02	6.2±0.07
Staphylococcus aureus ATCC 6538	7.6±0.08	7.7±0.07	7.7±0.09	8.9±0.08	7.4±0.06
Staphylococcus aureus CCM 2877	6.0±0.02	6.0±0.05	6.1±0.03	7.1±0.04	6.9±0.01
Streptococcus pyogenes ATCC 19615	7.8±0.02	7.9±0.01	7.9±0.04	8.2±0.04	8.2±0.05
Streptococcus pyogenes CCM 8112	6.0±0.09	6.0±0.07	6.5±0.08	7.2±0.07	7.0±0.04

Thus, for *Escherichia coli* ATCC10536 reference strains, the inhibition values reached a minimum of 6.2 ± 0.02 mm for a 5 ppm colloidal silver solution and a maximum of 8.8 ± 0.04 mm for a 20 ppm colloidal silver solution, compared to *Escherichia coli* CCM 2371 strain, which was not inhibited by the 5 ppm and the 10 ppm colloidal silver solutions, but presented maximum sensitivity of 6.4 ± 0.01 mm to 0.1N $AgNO_3$ solution. The *Klebsiella pneumoniae* ATCC 700603 strain showed little sensitivity

when using 5 ppm colloidal silver, only 6.7 ± 0.03 mm, increasing to 7.2 ± 0.07 mm in the case of 10 ppm concentration, to 7.8 ± 0.02 mm against 15 ppm concentration and a maximum of 8.2 ± 0.08 mm for 15 ppm concentration. Very similar values were found when using an 0.1N $AgNO_3$ solution, which resulted in values of 8.1 ± 0.09 mm. Compared to the results above, in the case of the *Klebsiella pneumoniae* CCM 1986 nosocomial strain, we noticed null values for 5 ppm, 10 ppm, and 15 ppm silver colloidal solutions and a maximum of 6.2 ± 0.01 for 20 ppm concentrations and 0.1N $AgNO_3$ solution.

Pseudomonas aeruginosa ATCC 25668 presented sensitivity in all the mentioned cases, as the diameter varies between 6.8 ± 0.01 mm and 7.9 ± 0.01 mm, compared to the nosocomial strain *Pseudomonas aeruginosa CCM 8912,* which did not react to 5 ppm and 10 ppm concentrations; the inhibition diameter only reached 6.7 ± 0.05 mm in the case of a colloidal silver concentration of 20 ppm, and 6.8 ± 0.08 mm when exposed to the 0.1N $AgNO_3$ solution.

Salmonella typhi ATCC 14028 was inhibited on much wider diameters than the other strains, as the values determined were situated between 7.0 ± 0.02 mm and 8.5 ± 0.04 mm; on the other hand, the nosocomial strain *Salmonella typhi CCM 8912* showed no sensitivity to 5 ppm and 10 ppm colloidal silver, slight sensitivity to a concentration of 15 ppm and 0.1N $AgNO_3$, and reached a maximum diameter of 7.5 ± 0.02 mm when a concentration of 20 ppm was used.

Great sensitivity was observed in the case of reference strains *Staphylococcus aureus* ATCC 6538 and *Streptococcus pyogenes* ATCC 19615, which were inhibited by low concentrations of colloidal silver: minimum values were 7.6 ± 0.08 mm, respectively 7.8±0.02 mm, and maximum values were 8.9 ± 0.08 mm, respectively 8.2 ± 0.04 mm. Conversely, nosocomial strains corresponding to *Staphylococcus aureus* CCM 2877 and *Streptococcus pyogenes* CCM 8112 only showed sensitivity to colloidal silver concentrated to 20 ppm, reaching a diameter of 7.1 ± 0.04 mm, respectively 7.2 ± 0.04 mm.

CONCLUSIONS

Considering the results obtained, we can state that nosocomial strains are very resistant to various concentrations of colloidal silver, while, in the case of low concentrations, the effect is null. *Salmonella typhi CCM 8912, Staphylococcus aureus CCM 2877* and *Streptococcus pyogenes CCM 8112* responded best to 20 ppm concentrations and to the 0.1N $AgNO_3$ solution. The most sensitive strains were the reference strains, especially *Pseudomonas aeruginosa* ATCC 25668, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus ATCC 6538* and *Streptococcus pyogenes* ATCC 19615, which showed significant inhibition zones. It is worth mentioning that a higher concentration of colloidal silver can have beneficial antibacterial effects, and these solutions are recommended to this end.

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