Silver nanoparticles as an antibacterial agent: a literature review

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Abstract

Growing bacterial resistance to antibiotics represents a serious threat to global health. In this context, silver nanoparticles (AgNPs) have emerged as promising alternatives due to their potent antimicrobial effect and ability to inhibit biofilm formation. This study aims to evaluate the potential of AgNPs as antibacterial agents through a theoretical analysis. A qualitative systematic review was conducted on silver nanoparticles, focusing on their antimicrobial properties, using inclusion and exclusion criteria to select 27 relevant articles from the BVS and PubMed databases. The antibacterial activity of AgNPs is influenced by factors such as size, shape and stability, and they are effective at disrupting bacterial membranes and biofilms via the release of silver ions and the generation of reactive oxygen species (ROS), as well as depending on controlled conditions to optimize their efficiency and avoid aggregation. The findings indicate that AgNPs represent a promising alternative in the fight against bacterial infections, contributing significantly to the advancement of research in this area.

Keywords: silver nanoparticle, antibacterial activity, mechanism of action, aggregation.

Nanopartículas de prata como agente antibacteriano: uma revisão de literatura

Resumo

A crescente resistência bacteriana a antibióticos representa uma grave ameaça à saúde global. Neste contexto, as nanopartículas de prata (AgNPs) emergem como promissoras alternativas, devido ao seu potente efeito antimicrobiano e capacidade de inibir a formação de biofilmes. Este estudo visa avaliar o potencial de AgNPs como agente antibacteriano, por meio de análise da fundamentação teórica. Este trabalho realiza uma revisão sistemática qualitativa sobre nanopartículas de prata com foco em suas propriedades antimicrobianas, utilizando critérios de inclusão e exclusão para selecionar 27 artigos relevantes das bases de dados BVS e PubMed. As AgNPs têm sua ação antimicrobiana influenciada por fatores como tamanho, formato e estabilidade, sendo eficazes na destruição de membranas bacterianas e biofilmes por meio da liberação de íons de prata e geração de espécies reativas de oxigênio, além de dependerem de condições controladas para otimizar sua eficiência e evitar agregação. Os resultados obtidos evidenciam que as AgNPs representam uma alternativa promissora no combate às infecções bacterianas, contribuindo significativamente para o avanço da pesquisa nessa área.

Palavras-chave: nanopartículas de prata, atividade antibacteriana, mecanismo de ação, agregação.

1. Introduction

Silver nanoparticles (AgNPs) are agglomerates of silver atoms between 1 and 100 nanometers in size, exhibiting various shapes and properties (Miranda, 2023). Their characteristics, which can be modulated by chemical, physical or ecological synthesis techniques (Wahab et al., 2021a), make them promising in fields such biomedicine, dentistry, textiles and water treatment, especially their antimicrobial potential (Bélteky et al., 2021). Their optical, chemical and biological aspects of AgNPs, including their high surface area relate to volume, ability to rupture cell membranes, generate reactive oxygen species (ROS), interrupt cellular respiration and interfere with DNA and protein molecules (Nqoro; Taziwa, 2024) give AgNPs remarkable efficacy against microorganisms.

In this context, in 2016, the UN General Assembly published a report warning of a worrying scenario: if the improper and indiscriminate use of antibiotics continues to grow, infections caused by multidrug-resistant bacteria will become the leading cause of death worldwide (Kraker et al., 2016; Panáček et al., 2021), so the exponential need to combat the proliferation of pathogenic microorganisms drives the search for alternatives to traditional antibiotic therapy (Michailidu et al., 2022).

Bacterial resistance to antibiotics occurs through main mechanisms such as genetic mutations, plasmid transfer, production of inactivating enzymes and modifications to the structure of the cell membrane (Teixeira et al., 2019). This process is accelerated by the inappropriate use of antibiotics, including overuse, self-medication and incomplete treatment, by selecting the most resistant microorganisms to survive, where bacteria that are sensitive to the drug are eliminated, while the resistant ones continue their proliferation (Santos, 2004).

This makes it necessary to develop an innovative drug that combines safety, biocompatibility and cost-effectiveness. It must be effective in combating bacterial cells without causing damage to healthy cells in the body, minimizing or eliminating side effects. To this end, silver nanoparticles have been shown to be able to combine the aforementioned effects by showing potential in biofilm inhibition, which is a virulent attribute of bacteria, as they interact with the components of the extracellular matrix, disorganizing and dispersing bacterial cells, facilitating the access of other molecules to the interior of the biofilm. In addition, they release Ag^+ ions which penetrate bacterial cells, inhibiting the function of proteins and DNA, leading to cell death. This process is enhanced by the generation of reactive oxygen species (ROS) which cause oxidative damage and by interfering with bacterial cellular respiration, reducing ATP production (Chopra, 2007; Kamradgi et al., 2022).

Several studies have investigated the use of silver nanoparticles in the fight against microorganisms due to the use of silver since antiquity and its high scientific and technological interest (Soldera et al., 2021), due to its characteristics that result in bacterial death or retardation (Siegel et al., 2020). This research proposal differs from previous ones by presenting a comprehensive approach, combining relevant knowledge for the development of safe and effective AgNPs, with great potential for advancing medicine and public health.

The aim of this research is to evaluate the potential of AgNPs as an antibacterial agent by analyzing the theoretical basis.

2. Materials and Methods

This work aims to conduct a systematic review of qualitative literature. This review method, known as the systematic approach, allows for the aggregation of information from various studies addressing the same therapy or intervention, regardless of whether the results are similar or different (Sampaio; Mancini, 2007). Qualitative research is defined by thorough analysis, systematic observation, detailed description and holistic interpretation, aiming to provide a deep and contextualized understanding of the subject bein studied (Gil, 1999).

To gather comparative and descriptive data, the Descriptors in Health Sciences (DeCS) tool was employed to define the following keywords: “colloidal silver”, “silver nanoparticle”, “antimicrobial”, “antibiotic”, “mechanism”, “action” and “effect”. Boolean operators were then applied to form the search expression: (“colloidal silver” OR “silver nanoparticle”) AND (“antimicrobial” OR “antibiotic”) AND (“mechanism” OR “action” OR “effect”), in the Virtual Library of the Ministry of Health (VHL) and National Library of Medicine (PubMed) databases.

Inclusion criteria were based on publication time (2019 to 2024); language (English or Portuguese); availability of the full text, and accessibility without restrictions.

Exclusion criteria were applied to eliminate references that were irrelevant to the focus of this research, such as studies on other nanoparticles, conjugated particles, sanitary, dental and cosmetic applications, or the use of AgNPs as antifungal and anticancer agents, which diverged from the primary theme. Paid-for articles and book chapters were not included. To this end, the titles and then the abstracts of the selected articles were analyzed.

First, filters for expression, publication time, language and availability were applied, resulting in 195 articles from VHL database and 87 from PubMed, yielding a total of 282 articles. Duplicate articles were removed during a screening process, leaving a total of 274 articles. During the title analysis, 113 articles were excluded, leaving 161 articles. Following the abstract review, 27 articles were identified, all of which were selected after a thorough reading of the full text. To visually and pedagogically illustrate the process of collecting bibliographic material, a flowchart was created using the Lucidchart platform, as shown in Figure 1.

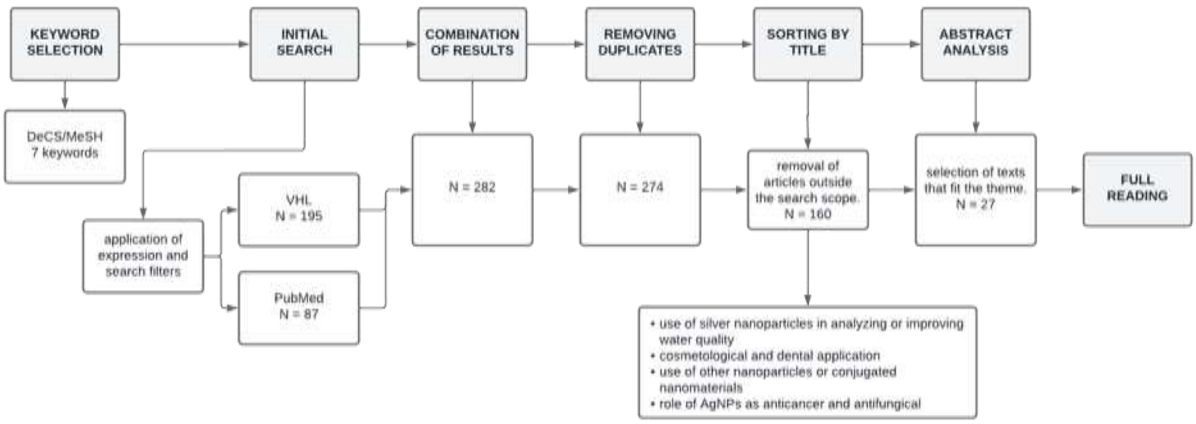

Figure 1. Flowchart of the process of obtaining bibliographic material. Source: Authors, 2024.

3. Results

The analyzed studies indicate that structural parameters of AgNPs, such as size, shape and surface area, which are linked to stability, mechanisms of action and toxicity, are crucial for developing AgNPs effective for antibacterial activity. By evaluating the significance of these parameters, it is possible to conclude that:

The size of the nanoparticles directly impacts their surface area, which in turn affects their antimicrobial activity (Nqoro; Taziwa, 2024). AgNPs sized between 10 to 15 nm are reported to be more effective against microorganisms, as they form pores in the bacterial cell membrane, facilitating nanoparticle penetration (Wahab et al., 2021b). Thus, smaller nanoparticles typically exhibit a larger surface area, leading to enhanced activity. However, Bélteky et al. (2021) note that extremely small nanoparticles tend to aggregate more, resulting in a loss of their activity. The reaction temperature influences the size of the AgNPs (Michailidu et al., 2022).

The shape of the AgNPs affects their interaction with the surrounding environment, influencing their stability and biodistribution and antibacterial activity. For example, nanoparticles with sharp points, such as triangular-shaped AgNPs, exhibit higher reactivity, as noted by Ahmad et al. (2021).

The methods used to synthesize AgNPs influence their size, shape, aggregation and mechanisms of action against bacteria (Michailidu et al., 2022). There are various synthesis methods, including physical routes that use mechanical force, laser radiation or temperature changes; chemical synthesis, which requires silver ions sources, solvents, reducing agents and stabilizers; and green synthesis, which uses biological resources to create AgNPs eliminating the toxic agents of the chemical methods (Wahab et al., 2021a).

The mechanisms of action must be broad-spectrum, meaning they should be effective against a wide variety of microorganisms. Employing multiple mechanisms of action is beneficial, combining strategies such as cell membrane damage, enzyme inhibition and DNA interactions to enhance efficacy and reduce resistance development (Kamradgi et al., 2022; Nqoro; Taziwa, 2024).

It is crucial that AgNPs are effective against microorganisms while maintaining low toxicity to human cells. They must be safe for both human and environmental use. A controlled and gradual release of silver ions can enhance efficacy and reduce toxicity, where the concentration and contact time correlate with the antibacterial action of AgNPs (Torky et al., 2022).

Most of the articles selected for this research did not address the storage conditions of the AgNPs tested. AgNPs must remain stable over time and under various storage conditions, which depend on agents that protect against aggregation (Badmus et al., 2020). It is important to consider pH and the presence of bio or macromolecules during AgNPs synthesis to prevent particle sedimentation over time (Bélteky et al., 2021; Rónavari et al., 2021).

The minimum inhibitory concentration (MIC), which measures the effectiveness of AgNPs against various microorganisms, determines the lowest concentration capable of inhibiting bacterial growth (Embrapa, 2009). The rate at which AgNPs kill microorganisms is another important factor, referred to as bacterial death kinetics

(Urodkova et al., 2023). Combining AgNPs with other compounds can enhance their effectiveness and slow bacterial development, creating a synergistic effect (Verma et al., 2013; Wahab et al., 2021a).

Based on these criteria for evaluating AgNPs efficacy, we have created a table summarizing the selected articles and the results obtained by the researchers, as shown in Table 1.

Table 1. Summary of selected articles, citation information and results.

Article title	Quote	Size and shape of AgNPs	Mechanism of action	Bacteria tested	Effectiveness
Nannochloropsis Extract-Mediated Synthesis of Biogenic Silver Nanoparticles, Characterization and In Vitro Assessment of Antimicrobial, Antioxidant and Cytotoxic Activities.	Gnanakani et al., 2019.	Average 57.25 nm; spherical and crystals with cubic and hexagonal symmetry.	It may be related to the penetration of silver ions through the formation of cavities in the bacterial cell membrane, in addition to the oxidative stress generated by ROS that denature proteins and the DNA of bacteria.	*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis.*	Efficacy is proven by observing the increase in the diameter of the inhibition of each bacterium used. The method used was cultivation in Mueller-Hinton broth and sowing on Mueller-Hinton agar.
Physiological response to silver toxicity in the extremely halophilic archaeon Halomicrobium mukohataei.	Buda et al., 2019.	Not reported, the research focuses on the physiological response of *Halomicrobium mukohataei* to silver.	Not reported.	Not reported.	Not reported.
Silver nanoparticles: aggregation behavior in biorelevant conditions and its impact on biological activity.	Bélteky et al., 2019.	10 nm; spherical.	It indicates that colloidal stability is essential for biological activity, where AgNPs interact with the structure of microorganisms.	*Bacillus megaterium* SZMC6031 and *Escherichia coli* SZMC0582.	The inhibitory power of the nanoparticles decreased as their degree of aggregation increased.
Biofilm Eradication Using Biogenic Silver Nanoparticles.	Estevez et al., 2020.	Not reported.	Not reported, but Raman spectroscopy shows reduction of DNA, amino acids and fatty acids, which may be due to AgNPs.	*Escherichia coli*.	Not reported.

Optomechanical Processing of Silver Colloids: New Generation of Nanoparticle-Polymer Composites with Bactericidal Effect.	Siegel et al., 2020.	Between 20 and 25 nm; spherical.	It indicates the release of silver ions, oxidative damage and interaction with the bacterial cell membrane.	*Escherichia coli* and *Staphylococcus epidermidis*.	It shows almost no efficacy against the bacteria tested.
Photo-assisted bio-fabrication of silver nanoparticles using Annona muricata leaf extract: exploring the antioxidant, anti-diabetic, antimicrobial, and cytotoxic activities.	Badmus et al., 2020.	35 nm; polycrystalline.	It is suggested that there is an induction of ROS, which capture electrons from the lipids of the bacterial cell membrane, lysing it.	*Staphylococcus aureus*, *Escherichia coli*, *Serratia marcescens*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella enterica*.	It was effective against all the bacteria mentioned, but the least sensitive were *B. cereus* and *S. enterica*.
Recent developments in biomolecule-based nanoencapsulation systems for antimicrobial delivery and biofilm disruption.	Vidallon; Teo, 2020.	Not reported.	Not reported.	Not reported.	Not reported.
Are Smaller Nanoparticles Always Better? Understanding the Biological Effect of Size-Dependent Silver Nanoparticle Aggregation Under Biorelevant Conditions.	Bélteky et al., 2021.	The aggregation behavior and biological activity of citrate-coated AgNPs, with diameters of 10, 20 and 50 nm; spherical, were investigated.	Does not specify.	*Bacillus megaterium* and *Escherichia coli*.	AgNP-I is not toxic to *B. megaterium* and *E. coli* after 6 hours. AgNP-II is not toxic to *B. megaterium* and *E. coli* after 24 hours and 6 hours of incubation. AgNP-III is 60% effective for *B. megaterium* and 70% for *E. coli*. It reports that increasing the size of AgNPs significantly reduces their tendency to aggregate in biological systems.
Insight into single-element nobel metal anisotropic silver	Ahmad et al., 2021.	Between 26 and 138 nm; triangular, cubic	It describes that the sharp tips of AgNPs were crucial for the physical deterioration of	*Escherichia coli* and *Staphylococcus*	Three shapes of AgNPs were used: triangle, cube and rod. The most effective against bacteria was the rod

nanoparticle shape-dependent selective ROS generation and quantification.		and rod-shaped.	bacterial morphology. The synergy with ROS enhanced the adhesion of the NPs to the cell membrane, resulting in a superior and multifactorial bactericidal effect compared to the authors' reviews.	*aureus*.	shape, the second most effective was the cube and the third, but still effective, was the triangle.
Polyvinyl-Pyrrolidone-Coated Silver Nanoparticles-The Colloidal, Chemical, and Biological Consequences of Steric Stabilization under Biorelevant Conditions.	Rónavari et al., 2021.	Between 6 and 12 nm; spherical.	Not reported. Describes the importance of the stability of AgNPs, as lack of attention to this parameter affects therapeutic efficacy.	*Bacillus megaterium* and *Escherichia coli*.	Cells that were not treated with NaCl had lower cell viability when exposed to AgNPs. On the other hand, cells that were treated with NaCl and then exposed to AgNPs, toxicity was not influenced by the loss of stability; there was only a slight variation in the intensity of the toxic effect.
Silver Covalently Bound to Cyanographene Overcomes Bacterial Resistance to Silver Nanoparticles and Antibiotics.	Panáček et al., 2021.	Entre 4 a 8 nm; not reported.	It indicates that AgNPs bind to membrane proteins, altering their structure and function, as well as producing ROS, disrupting the bacterial structure.	*Escherichia coli* and *Pseudomonas aeruginosa*.	It is effective, capable of being strong even against intensely resistant strains.
Silver Micro-Nanoparticle-Based Nanoarchitectures: Synthesis Routes, Biomedical Applications, and Mechanisms of Action.	Wahab et al., 2021. (a)	Non-specifically, it suggests that NPs of up to 10 nm are more efficient.	It reviews that the mode of action of AgNPs against pathogens is influenced by factors such as the physicochemical properties of the nanoparticles (size, shape, charge), interactions with the environment (aggregation, dissolution), production of ROS and interactions with different materials.	Not reported. Review article citing bacteria: *Escherichia coli* and *Staphylococcus aureus*.	Review article that reports that it is effective, using a qualitative method and visualizing the presence of the inhibition zone.

Silver Nanoparticle-Based Nanocomposites for Combating Infectious Pathogens: Recent Advances and Future Prospects.	Wahab et al., 2021. (b)	Non-specific, it suggests that NPs between 1 and 10 nm are more reactive; it suggests that spherical NPs have a high atomic density of facets and a high surface-to-volume ratio.	It reviews that AgNPs induce cell death through mechanisms that involve interaction with biomolecules rich in sulphur and phosphorus, such as DNA, and disruption of the structure of the bacterial cell membrane.	Article revised using *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*.	It mentions that smaller, spherical AgNPs are more effective on *P. aeruginosa* and *E. coli*. The rest of the bacteria cited were used in another article which concludes that AgNPs with a size of less than 10 nm have the most remarkable ability to bind to the sulfur-containing membrane, thus possessing greater permeability through the membrane and greater ability to cause bacterial cell death.
Surface Minimal Bactericidal Concentration: A comparative study of active glasses functionalized with different-sized silver nanoparticles.	Barzan et al., 2021.	6, 30 and 52 nm; spherical.	Even if the AgNPs are small in size, if they agglomerate their activity will be compromised. The main effect is reported to be the release of silver ions and contact with bacteria.	*Escherichia coli* ATCC 8739.	By reducing the agglomeration that occurs in liquids, the antibacterial efficacy of AgNPs has been improved.
Characterization of Talaromyces islandicus-mediated silver nanoparticles and evaluation of their antibacterial and anticancer potential.	Kamradgi et al., 2022.	Between 13 and 66 nm; spherical.	It indicates that the possible mode of action is through binding to the bacterial cell membrane, which generates osmotic imbalance and the leakage of cellular constituents.	*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*	The method used to visualize efficacy was the zone of inhibition, with AgNP achieving significant inhibition against *P. aeruginosa*, *E. faecalis*, *E. coli*, *S. aureus* and *B. subtilis*.
Genetic Relatedness, Antibiotic Resistance, and Effect of Silver Nanoparticle on Biofilm	Ahmed et al., 2022.	45 nm; not reported.	Due to the high surface to volume ratio, it is proposed that AgNPs act as exopolysaccharide (EPS)	*Clostridium perfringens*.	It is effective, inhibiting the biofilm of the bacteria tested.

Formation by Clostridium perfringens Isolated from Chickens, Pigeons, Camels, and Human Consumers.			disruptors, the biofilm.		
Silver nanoparticle effect on Salmonella enterica isolated from Northern West Egypt food, poultry, and calves.	Torky et al., 2022.	Between 26,5 and 45 nm; spherical.	Adherence and penetration of AgNPs inside bacteria, forming cavities in the membrane.	*Salmonella spp*.	An in vivo study found that AgNPs proved to be effective preventive and therapeutic agents, due to their excellent physical mechanism of action.
Silver nanoparticle incorporation into flexible polyamide 12 membranes.	Souza et al., 2022.	Between 35 and 50 nm; crystalline.	By interacting with the bacterial cell membrane, AgNPs induce the denaturation of membrane proteins, compromising cell permeability and leading to a loss of structural integrity, due to the high affinity of AgNPs with functional groups such as phosphate, hydroxyl, amine and thiol.	Not reported. A PA12 membrane was used instead of bacteria.	Not reported. Test carried out on polyamide membrane.
Silver Nanoparticle Production Mediated by Vitis vinifera Cane Extract: Characterization and Antibacterial Activity Evaluation.	Michailidu et al., 2022.	Between 1 and 30 nm; spherical.	Interaction with silver ions to inhibit biofilm and lyse bacterial cells.	*Pseudomonas aeruginosa* PAO1, ATCC 10145, ATCC 15442, DBM 3081 and DBM 3777.	It is effective, this type of AgNPs is able to suppress planktonic cells of all the strains used and biofilm inhibition has also occurred.
Silver Nanoparticle-Based Therapy: Can It Be Useful to Combat Multi-Drug Resistant Bacteria?	Mateo; Jiménez, 2022.	Suggests that 5 to 30 nm are more effective against bacteria; not specified.	They induce cell death through destabilization of the plasma membrane, inhibition of gene replication and expression and	Not reported. Review article citing *Acinetobacter baumannii*,	Not reported. Review article that mentions how the mechanisms of action of AgNPs act on multiple levels. They increase the permeability of the plasma

			mitochondrial dysfunction. Permeabilization of the membrane leads to the release of cellular content, while inhibition of the cellular machinery prevents protein synthesis and energy production. The generation of ROS promotes the oxidation of biomolecules, accelerating cell deterioration.	*Pseudomonas aeruginosa*, *Enterobacteriaceae* and MDR *Staphylococcus aureus*.	membrane, resulting in the release of intracellular contents and cell death. In addition, they interrupt DNA replication, inhibit the expression of ribosomal subunits, and inactivate proteins and enzymes. AgNPs also alter the function of membrane respiratory enzymes and promote the formation of ROS, causing oxidative damage to cell contents.
Silver nanoparticles synthesized from the seaweed Sargassum polycystum and screening for their biological potential.	Thiurunavukkarau et al., 2022.	Less than 100 nm; spherical.	Not reported.	*Escherichia coli* MTCC1687, *Bacillus subtilis* MTCC441, *Klebsiella pneumoniae* MTCC4030, *Staphylococcus epidermidis* MTCC435, *Vibrio cholera* MTCC0139, *Pseudomonas fluorescens* MTCC664, *Micrococcus luteus* MTCC4821, *Staphylococcus aureus* MTCC 96, e *Serratia marcescens* MTCC86.	It is effective, showing a zone of inhibition for the bacteria tested.
Tryptone-stabilized silver nanoparticles' potential to mitigate planktonic and biofilm growth forms of	Pandey et al., 2022.	18 (AgNPs) and 170 nm (Ts-AgNPs); spherical.	Modification of cell surface hydrophobicity by Ts-AgNPs, associated with inhibition of biofilm formation. The	*Serratia marcescens*.	It is effective, using the spherical morphology AgNP is able to show strong antibacterial activity, such as altering the hydrophobicity of the cell

Serratia marcescens.			diffusion of the NPs in the EPS layer, the generation of ROS and the reduction of cell motility suggest that they affect the adhesion and dispersion of bacterial biofilms.		surface and inhibiting biofilm formation.
Vaterite vectors for the protection, storage and release of silver nanoparticles.	Ferreira et al., 2022.	From 7 to 13 nm; spherical.	AgNPs are capable of disrupting the cell membrane and generating ROS.	*Escherichia coli* O157:H7, *Staphylococcus aureus* methicillin-resistant *and Pseudomonas aeruginosa* PA01.	The CaCO3/AgNPs-PVP hybrid is effective in eliminating the bacteria mentioned through the action of AgNPs.
A whole cell fluorescence quenching-based approach for the investigation of polyethyleneimine functionalized silver nanoparticles interaction with Candida albicans.	Tiwari et al., 2023.	Between 4.4 and 6.8 nm; spherical.	Not reported.	Not reported.	Not reported.
Colloidal Silver Nanoparticles Obtained via Radiolysis: Synthesis Optimization and Antibacterial Properties.	Miranda et al., 2023.	20 nm; spherical.	They can cause damage by releasing silver ions and generating ROS, which interrupt the metabolism, DNA replication and synthesis of proteins and enzymes in bacteria, leading to cell death.	*Staphylococcus aureus*.	Effective in inhibiting at a concentration of 0.6 μg·mL-1 and effective in eradicating the bacteria at a concentration of 5.6 μg·mL-1.
Formation Kinetics and Antimicrobial Activity of	Urodkova et al., 2023.	Between 68 and 77 nm; polycrystalline.	Not reported.	*Escherichia coli*, *Pseudomonas*	The method used to visualize efficacy was serial dilution, showing significant

Silver Nanoparticle Dispersions Based on N-Reacetylated Oligochitosan Solutions for Biomedical Applications.				*aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus*.	action against the bacteria tested.
Silver nanoparticle technology in orthopaedic infections.	Jeyaraman et al., 2023.	10 nm.	It reviews that metal ions interact with DNA, while AgNPs penetrate cells, inhibiting ATP production and bacterial growth, inducing oxidative stress and dispersing biofilms.	Not reported. Review article that cites several bacteria, but does not focus on them.	Not reported. Review article aimed at preventing the colonization of bacteria on stainless steel using AgNPs.

Source: Authors, 2024.

4. Discussion

4.1 Bacterial cell walls and the action of AgNPs

The bacterial cell wall performs essential functions, such as protecting intracellular components from external factors, preventing cell rupture when intracellular water levels exceed extracellular levels, and maintaining bacterial shape, including supporting flagella, when present. In addition to these structural and protective functions, the cell wall also contributes to the bacteria's ability to cause virulence factors (Tortora et al., 2016). A notable example is endotoxin, a lipopolysaccharide (LPS) present in the cell wall of certain Gram-negative bacteria, which is released in small quantities during normal metabolism (Lourenço et al., 2005). The cell wall may also contain lipids that make it easier for the bacteria to enter and remain in the human body. One example is the mycolic acid present in the cell wall of *Mycobacterium tuberculosis* (Petrilli, 2019).

The membrane of a bacterium is made up of a cell wall, which has a macromolecular network called a peptidoglycan. This network is formed by combining a repetitive disaccharide with a polypeptide. There is an important difference between Gram-positive and Gram-negative bacteria: the former generally have several layers of peptidoglycan, while the latter have only one or a few layers of this substance (Madigan et al., 2016).

In Gram-positive bacteria, in addition to having multiple layers of peptidoglycan, proteins and two distinct types of teichoic acid are found. The first type, lipoteichoic acid, crosses the peptidoglycan layer and binds to lipids in the plasma membrane. The second type is wall teichoic acid, which is directly linked to the peptidoglycan layer. These acids play an important role in cell growth and the regulation of autolysins. Gram-negative bacteria have one or a few peptidoglycan layers, which makes them more susceptible to mechanical disruption. They also lack the acids mentioned above. Lipopolysaccharides are the originators of the antigenic response in Gram-negative bacteria, just as proteins make up their plasma membrane (Trabulsi; Alterthum, 2015).

Of the 23 bacteria analyzed in the selected articles - including bacteria used in experimental research and those used in theoretical studies - 12 of the species are Gram-positive and 11 species are Gram-negative. When considering Gram staining to analyze the antibacterial effect of AgNPs, the reports are quite contradictory. Viana et al (2021) suggest that AgNPs inhibit the growth of Gram-positive bacteria more, while Brito et al. (2017) point out that AgNPs affect the development of Gram-negative bacteria more.

Despite this, the effect of AgNPs is observed, which depends on other aspects such as the format, size and concentration of the solution tested, as evidenced by the study by Siegel et al. (2020), Badmus et al. (2020) and Panáček et al. (2021), which show different results on *E. coli* bacteria, for example.

4.2 Size and shape in the action of AgNPs

Physical characteristics, such as the size and shape of AgNPs, significantly influence their antibacterial efficiency. From the analysis of the articles, it was observed that smaller nanoparticles have a greater surface area in relation to volume. This is because the smaller size makes it easier to pass through cell membranes, which can lead to a higher concentration of silver ions inside the cells and, consequently, a more powerful antimicrobial effect (Sanfelice et al., 2022). In addition, the shape of the nanoparticles can influence their interaction with different bacterial cell components and their penetration efficiency. For example, triangular AgNPs can have a larger and more reactive surface area (Ahmad et al., 2021). Although there is no universal “ideal” size for all bacteria, smaller AgNPs, between 10 and 15 nm, are generally more effective, as described by Wahab et al. (2021b).

Among the selected studies, the production of AgNPs ranging from 1 to 170 nm was reported, with the description of square, cylindrical, spherical, rod and triangular shapes. Of the 27 articles, 14 report spherical AgNPs; 9 do not report size and/or shape and the remaining 4 articles report different shapes, such as rods or triangles. Figure 2 shows the spherical, triangular and rod shapes of AgNPs in an electron microscopy test.

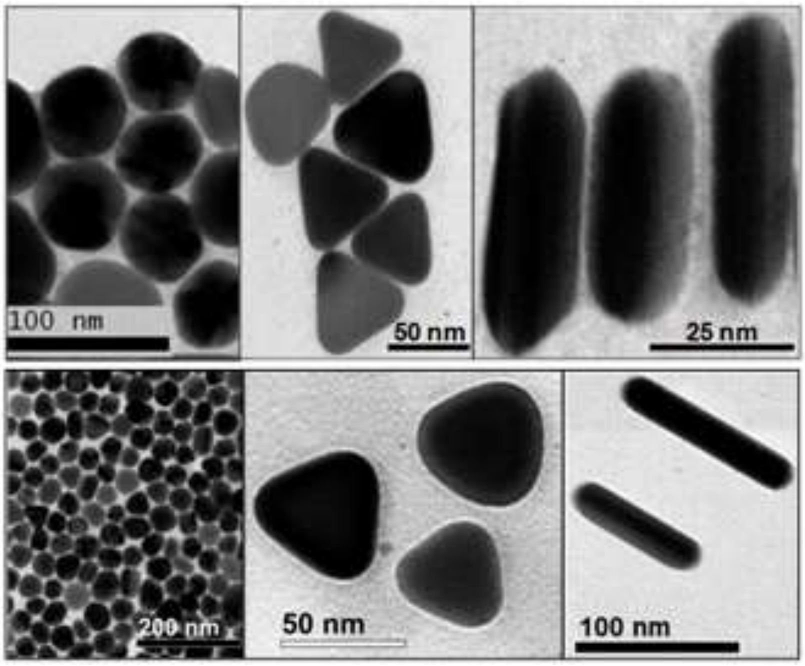

Figure 2. Demonstration of AgNPs shapes by electron microscopy. Source: Adapted from Tak et al., (2015). Open access article distributed under the terms of the Creative Commons Attribution 4.0 International License: http://creativecommons.org/licenses/by/4.0.

The synthesis process and modification techniques affect the size, shape, surface, distribution and reactivity of AgNPs, impacting on their efficacy and safety. The main approaches can be classified into three categories: physical synthesis, where AgNPs are obtained through the mechanical fragmentation of larger particles. Techniques such as grinding and abrasion are used, allowing a high degree of control over the shape and size of the particles; chemical synthesis, which involves chemical reactions in solution, where silver ions are reduced to metallic silver. The choice of chemical reagents used, such as reducing and stabilizing agents, influences the properties of the final AgNPs; and biological or green synthesis, being a more sustainable and environmentally friendly approach, uses extracts from plants or microorganisms to reduce the silver ions and stabilize the NPs. This methodology offers advantages such as lower environmental impact and the possibility of obtaining AgNPs with unique properties (Buda et al., 2019; Siegel et al., 2020; Wahab et al., 2021a; Kamradgi et al., 2022; Miranda et al., 2023).

4.3 Effectiveness and aggregation of AgNPs

Bélteky et al. (2021) describe that aggregation is a phenomenon that occurs when particles dispersed in a medium come together to form larger, more complex structures. This process is influenced by factors such as the force of attraction between the particles, the temperature and the concentration of the particles in the medium.

Michailidu et al. (2022) points out that the pH and temperature of the medium can influence the action of AgNPs in aspects such as stability and interaction with bacterial cells. Corroborating this idea, Ferreira et al. (2022) state that AgNPs show high instability when combined with any type of salt and Tris buffer solution (TBS) and Bélteky et al. (2021) demonstrate that a more acidic pH favors particle aggregation, while a more alkaline pH tends to inhibit or reduce aggregate formation.

Vidallon and Teo (2020) suggest that coating AgNPs with ligands or polymers enhances stability and prevents colloidal aggregation, a finding further corroborated by Souza et al. (2022). Surface modification can also be used to control the release of substances from AgNPs. By incorporating molecules of interest into the coating, it is possible to develop controlled release systems, in which the substance is released gradually and in a targeted manner, which would help in therapy against microorganisms (Ferreira et al., 2022).

Considering these factors is vital to guaranteeing the stability and functionality of AgNPs. The aggregation of these particles negatively impacts their properties, including their optical, thermal, electrical and antimicrobial properties, as described by Wahab et al. (2021a). This aggregation reduces the surface area available for interaction, hinders penetration into bacterial membranes and compromises the release of silver ions (Jeyaraman et al., 2023).

4.4 Mechanisms of antimicrobial action of AgNPs

The plasma membrane is a structure that defines the cell's boundary, separating the intracellular environment from the extracellular space. Its primary function is to act as a selective barrier, controlling the passage of substances and ensuring cellular homeostasis. This property is conferred by its composition, predominantly lipid and protein, which forms a fluid and dynamic bilayer. The membrane's selective permeability allows nutrients to enter and metabolic products to leave, while preventing the free diffusion of harmful substances (Tortora et al., 2016).

Membrane integrity is crucial for cell survival. Damage or alterations to its structure can lead to the loss of this barrier function, resulting in lysis and consequent cell death. Membrane dynamics, however, are essential for several bacterial cell functions, such as substance transport, signaling and cell adhesion (Madigan et al., 2016).

When AgNPs are used, the bacterial cell membrane is altered due to the penetration of silver ions (Ag^+), which form pores, leading to cell lysis, as reported in the studies by Gnanakani et al. (2019), Siegel et al. (2020), Barzan et al. (2021), Michailidu et al. (2022), Miranda et al. (2023) and Jeyaraman et al. (2023). In addition, these ions contribute to the generation of ROS, triggering oxidative stress. By capturing electrons from membrane lipids, silver ions alter bacterial structure, inhibit ATP production, interrupt genetic replication and promote greater adhesion of AgNPs to the bacterial surface (Gnanakani et al., 2019; Badmus et al., 2020; Ahmad et al., 2021; Panáček et al., 2021; Mateo; Jiménez, 2022; Ferreira et al., 2022; Miranda et al., 2023; Jeyaraman et al., 2023).

The adhesion of AgNPs to the cell membrane, influenced by their size and shape conformations, and by interactions with biomolecules containing sulphur, phosphorus, hydroxyl, amine or thiol, causes an osmotic imbalance, contributing to the dispersion of cell contents and a deeper interaction of the NPs with the bacterial cellular machinery (Ahmad et al., 2021; Wahab et al., 2021b; Kamradgi et al., 2022; Torky et al., 2022; Souza et al., 2022; Pandey et al., 2022).

Therefore, the mechanism of action of AgNPs as an antibacterial agent is shown through direct damage to the cell membrane, generation of ROS, adhesion and penetration of particles and interruption of the vital processes of bacteria.

Figure 3, drawn up in the Canva program, graphically demonstrates the mechanisms of action of AgNPs acting against the cell wall, DNA, proteins and enzymes of bacteria; the production of ROS; and in the dissociation of bacterial biofilm.

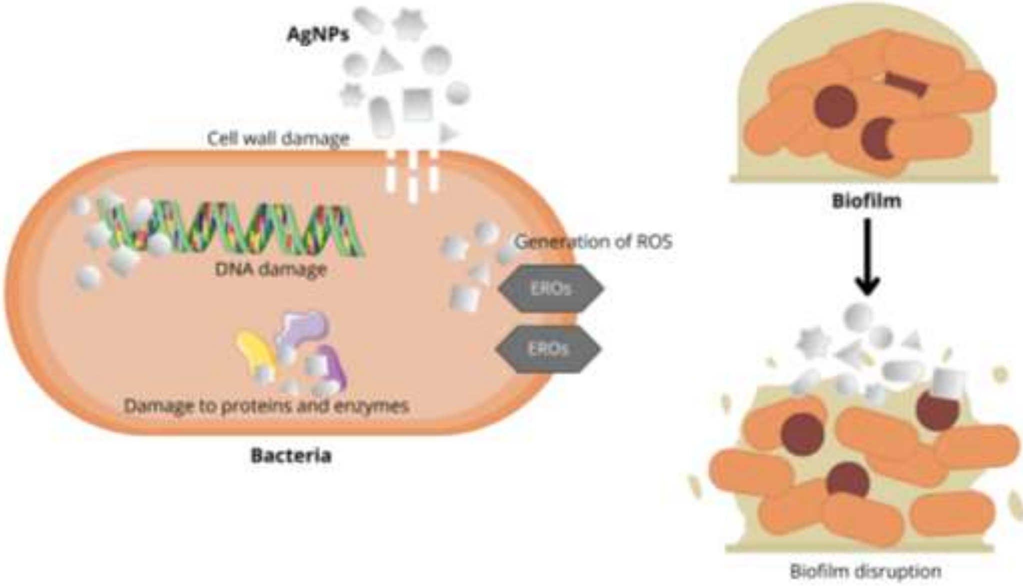

Figure 3. Representation of the mechanisms of action of AgNPs on bacteria and biofilm. Source: Authors, 2024.

4.5 Action of AgNPs against bacterial biofilm

Biofilm is a pathogenic characteristic, a microbial community that adheres to surfaces and is immersed in a self-produced extracellular polymeric matrix (EPS). This matrix, composed of polysaccharides, proteins and nucleic acids, provides an ideal microenvironment for microbial growth and persistence, facilitating adhesion to various surfaces and helping to retain nutrients and water (Madigan et al., 2016; Vidallon; Teo, 2020).

Biofilm formation provides bacteria with protection against antibiotics and disinfectants, making them more resilient to treatment. When integrated into this structure, bacteria are more tolerant to stress and less susceptible to antimicrobial agents due to the physical barrier imposed by the extracellular matrix and the exchange of resistance genes between different bacterial species (Bergamo et al., 2020; Ahmed et al, 2022). Antimicrobial resistance in the context of biofilms is different from intrinsic resistance. When dispersed, microorganisms can become susceptible to treatment again, making biofilm infections a therapeutic challenge that requires a comprehensive approach (Abrantes; Nogueiro, 2022).

Common strategies to combat biofilms consist of anti-infective surfaces and the combination of antimicrobial agents, where the combined use of different antibiotics and disinfectants can increase the effectiveness of the treatment (Aydos, 2016).

As highlighted by Ahmed et al. (2022), AgNPs act as transporters of substances that disrupt the EPS matrix. The author points to studies that report biofilm inhibition and reduction in Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Michailidu et al. (2022) report that AgNPs are able to suppress planktonic cells and inhibit biofilm formation in Pseudomonas aeruginosa through interaction with silver ions, corroborated by Jeyaraman et al. (2023).

Pandey et al. (2022) demonstrated that spherical AgNPs alter the hydrophobicity of the cell surface, hindering the initial adhesion of bacteria to the substrate and, consequently, biofilm formation.

The ability of AgNPs to both prevent biofilm formation and eradicate biofilms - either through interaction with the extracellular matrix with silver ions or by altering hydrophobicity - is relevant to the context of contamination, chronic infections and antimicrobial resistance.

4.6 Toxicity of AgNPs

The toxicity of AgNPs refers to their potential adverse effects on living organisms, ranging from microorganisms to humans and the environment. There may be toxicity to eukaryotic cells through the same mechanisms of action that affect bacteria, possibly inducing apoptosis and an inflammatory response. The magnitude of these effects depends on factors such as cell type, dose and time of exposure to AgNPs (Viegas, 2018; Durán et al., 2019).

Bélteky et al. (2021) indicate that smaller AgNPs showed greater toxicity to DU145 cells (prostate carcinoma epithelial cell) and HaCaT cells (human keratinocytes), when not treated with NaCl. This greater toxicity can be attributed to the mechanisms of action described above. However, pre-incubation with NaCl promoted the aggregation of AgNPs, reducing their toxicity. This observation is in line with the results of Tiwari et al. (2023) and Ronávari et al. (2021), who demonstrated that the colloidal stability of AgNPs is an important factor in maintaining their toxicity.

There are advantages to the use of AgNPs as an antibacterial agent due to their spectrum of action and efficacy, but the disadvantages are toxicity, environmental impacts due to the release of AgNPs into the environment and the possible induction of bacterial resistance. Although bacterial resistance to AgNPs is less common than to traditional antibiotics, studies indicate that some bacteria may develop resistance mechanisms, as pointed out by Durán et al. (2019) and Panáček et al. (2021).

4.7 Applications of AgNPs

Silver nanoparticles have shown great antimicrobial potential as described by Amna et al. (2021), boosting their application in various areas, especially in health and the environment.

In the field of orthodontics, AgNPs are used due to their antibacterial properties and are incorporated into various dental materials, such as adhesives, implants and relineers, with the aim of reducing the formation of bacterial biofilms and preventing infections, as described by Pignataro (2020); Kreutz et al. (2022), Pérez-Tanoira et al. (2022) and Dhingra et al. (2022).

The studies by Quintero-Quiroz et al. (2020) and Chih et al. (2023) provide evidence of the effectiveness of AgNPs in medical instruments, demonstrating that AgNPs can reduce bacterial contamination and prevent infections, when associated with stainless steel and polymers, for example.

AgNPs are used in wound healing dressing formulations, showing significant potential in the tissue regeneration process and in preventing infections. The approaches presented in the studies by Dehkordi et al. (2019), Yang et al. (2021), Ragothaman et al. (2021), Souza et al. (2022) and Jhumi et al. (2023) explore different combinations and methods of incorporating nanoparticles into gels, seeking to optimize their efficacy and safety for wound treatment.

Shen et al. (2019) and Lyu et al. (2022) demonstrate the possibility of combining AgNPs with other technologies, such as reduced graphene oxide scaffolds and superhydrophobic treatments, to create materials with the potential to reduce contamination and prevent the spread of pathogens in the environment. The studies by Weng et al. (2020) and Nqoro & Taziwa (2024) address the use of AgNPs in three-dimensional scaffolds to aid in the healing of wounds and infected bone defects. In the study by Turki et al. (2022), silver nanoparticles were used to evaluate stress and immune responses in the snail *Helix aspersa*, a species used as a bioindicator in the biomonitoring of silver nanoparticle contamination.

5. Conclusions

This article explores various aspects of silver nanoparticles in the fight against bacteria, including synthesis techniques, mechanisms of action, toxicity, and biofilm activity. After carefully analyzing the data, we conclude that AgNPs hold significant potential as antibacterial agents, contributing valuable insights to the existing body of knowledge. The results presented here provide a foundation for future research, driving the development of new strategies to combat the rising threat of antibiotic resistance.

We emphasize that developing effective and safe therapies based on silver nanoparticles requires in-depth studies on toxicity, dosage, body distribution, and large-scale production methods. This knowledge is crucial for ensuring the translation into clinical research and its subsequent application in the healthcare field.

6. Authors' Contributions

Author Débora and Thalyta contributed to the bibliographic search, reading, writing and translation of this study. Researcher Giovana helped with the writing and content review, together with researcher Stephanie, who contributed to the data analysis.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

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