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BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY - REVIEW

Antibacterial and antibiofilm potential of silver nanoparticles against antibiotic-sensitive and multidrug-resistant *Pseudomonas aeruginosa* strains

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Abstract

Due to the severity of infections caused by *P. aeruginosa* and the limitations in treatment, it is necessary to find new therapeutic alternatives. Thus, the use of silver nanoparticles (AgNPs) is a viable alternative because of their potential actions in the combat of microorganisms, showing efficacy against Gram-positive and Gram-negative bacteria, including multidrug-resistant microorganisms (MDR). In this sense, the aim of this work was to conduct a literature review related to the antibacterial and antibiofilm activity of AgNPs against antibiotic-sensitive and multidrug-resistant *Pseudomonas aeruginosa* strains. The AgNPs are promising for future applications, which may match the clinical need for effective antibiotic therapy. The size of AgNPs is a crucial element to determine the therapeutic activity of nanoparticles, since smaller particles present a larger surface area of contact with the microorganism, affecting their vital functioning. AgNPs adhere to the cytoplasmic membrane and cell wall of microorganisms, causing disruption, penetrating the cell, interacting with cellular structures and biomolecules, and inducing the generation of reactive oxygen species and free radicals. Studies describe the antimicrobial activity of AgNPs at minimum inhibitory concentration (MIC) between 1 and 200 μg/mL against susceptible and MDR *P. aeruginosa* strains. These studies have also shown antibiofilm activity through disruption of biofilm structure, and oxidative stress, inhibiting biofilm growth at concentrations between 1 and 600 μg/mL of AgNPs. This study evidences the advance of AgNPs as an antibacterial and antibiofilm agent against *Pseudomonas aeruginosa* strains, demonstrating to be an extremely promising approach to the development of new antimicrobial systems.

Keywords AgNPs · Antibacterial activity · Antibiofilm activity · *Pseudomonas aeruginosa* · Multidrug-resistant bacteria · Biofilm

Introduction

Bacterial infections represent one of the world's major public health problems, mainly due to increased persistence of these infections, treatment failure, and consequently, high rates of morbidity and mortality. It is estimated that 14% of hospital admissions are due to bacterial infections [1]. This problem is characterized mainly by bacterial resistance, which refers to the ability of bacteria to survive even after exposure to antimicrobials, and can happen by mechanisms such as reduced cellular permeability, enzymatic inactivation, production of flow pump, and alteration of binding site, in addition to biofilm production, which offers greater stability and safety to the microorganism [2].

In particular, the bacterium *Pseudomonas aeruginosa* has been increasingly gaining resistance to various antimicrobials, such as piperacillin/tazobactam, carbapenems, fluoroquinolones,

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ceftazidime, aminoglycosides, and polymyxins, and are already present in almost all continents of the world [3–8].

P. aeruginosa is a γ-proteobacterium, Gram-negative, non-fermentative, responsible for infection of a wide variety of organisms, including plants, animals and humans. Because it is an opportunistic pathogen, it is responsible for causing bacteremia, otitis, soft tissue infection, urinary tract, and respiratory infections [9]. Immunocompromised patients with pulmonary infection or burns are considered the risk groups for their colonization. In addition, there is still the ability to colonize implanted medical devices such as catheter [10, 11]. Once infection is established, *P. aeruginosa* progresses to a growth mode characterized as biofilm, by the formation of an extracellular matrix composed by exopolysaccharides, proteins and extracellular DNA [12, 13].

Due to adaptation, and exposure to various antibiotics, some strains become multiresistant to the therapies currently employed, especially carbapenems, and also adapt to the biofilm condition [14]. Thus, it is necessary to develop new therapeutic approaches able to exert not only antibacterial activity, but also antibiofilm since the latter is considered a challenge for eradication [15, 16]. Therefore, the use of materials on a nanometric scale is a viable strategy for carrying biomolecules and drugs, as it provides advantages such as increased half-life time and systemic circulation time, greater contact between the compound and the pathogen, better bioavailability, and greater absorption, resulting in a better adherence to therapy and more efficient treatment [17, 18].

Among the nanosystems, silver nanoparticles (AgNPs) are shown to be a potential application, because they present relevant physicochemical characteristics necessary to combat microorganisms, such as stability, colloidal state, and good chemical interaction [19]. AgNPs are small reduced particles of silver metal with high potential for biological application, and can present several forms, such as spherical, flat, triangular, tetrahedral, prismatic, cubic, octahedral and irregular, and variable size, with a range between 1 and 100 nm [20, 21]. Therefore, research related to the development or modifications of compounds with antibacterial and antibiofilm activities, especially against *P. aeruginosa* strains, is an area that interests and growth. In this sense, the present work aims to conduct a literature review related to the antibacterial and antibiofilm activity of AgNPs against pathogenic strains of *P. aeruginosa*.

This is a descriptive study of the literature review based on the following stages: identification of the theme and development of the guiding question; establishment of inclusion and exclusion criteria, analysis, and selection of studies; interpretation of data and results; presentation of the review. The guiding question was "What are the benefits of silver nanoparticles for the treatment of infections caused by *P. aeruginosa*?" The literary search took place from articles indexed in international virtual libraries, *U.S. National Library of Medicine* (PubMed), *ScienceDirect*, and *Scientific Electronic Library Online* (SciELO).

The inclusion criteria adopted were complete studies, published in English that were related to the proposed theme and are indexed in these databases, and published between 2011 and 2020. In turn, repeated studies, studies that do not address the proposed theme, incomplete studies, duplicates, monographs, and publications of events were excluded. The following descriptors were used: silver nanoparticles, AgNPs, antimicrobial properties/activity, antibiofilm properties/activity, and *P. aeruginosa*. From the search in the databases, 100 articles were selected using the inclusion and exclusion criteria. The analysis of the selected studies made it possible to identify variables, observations, and data that gathered the knowledge about the use of AgNPs against *P. aeruginosa*. Both the analysis and the relationship of the data extracted from the articles were developed descriptively, making it possible to count, observe, describe, and classify them, with the purpose of organizing the knowledge generated about silver nanoparticles.

Synthesis of AgNPs

AgNPs can be synthesized by different methods and present different characteristics according to these methods. The most used approaches are the synthesis through chemical methods that uses organic solvents and inorganic reducing agents. The main reagents are sodium citrate, ascorbate, sodium borohydride (NaBH4), elemental hydrogen, Tollens' and N, N-dimethylformamide (DMF) reagent, and stabilizing agents such as vinyl alcohol, polyvinylpyrrolidone, polyethylene glycol, polyacidomethacrylic, and polymethylmethacrylate [22, 23]. This method is based on the reduction of metal ions for the formation of atoms, and then these are aggregated in a controlled manner [22, 24, 25]. However, this synthesis can result in AgNPs with chemicals sedimented in their surfaces. Some of these chemicals can be toxic and harmful, and can increase AgNPs toxicity to human cells, making its use unfeasible [26].

The methodologies that involve the synthesis of AgNPs by physical methods include the technique of laser ablation and evaporation-condensation. In laser ablation, silver is introduced into a liquid environment that, in turn, undergoes radiation from a pulsed laser, which results in the formation of AgNPs [27]. However, the wavelength that the laser reaches the metallic target, the pulsation period of lasers, and the liquid medium are some of the factors that determine the effectiveness and characteristics of the nanoparticles. This technique is one of the methods that results in silver nanoparticles without using chemical reagents [28, 29].

Another approach used is evaporation-condensation. In this method, the silver is evaporated from the center of a tube

furnace to a gas phase route, allowing the synthesis of AgNPs at atmospheric pressure. Then, the products are handled on a nanometric scale, and through physical processes, the particles are broken down to the nanoscale. However, the tube oven occupies a large space and consumes high energy, thereby raising the ambient temperature of the metal source and requiring a long duration to maintain thermal stability [25, 29]. Besides that, this method results in imperfections on the surface structure of the AgNPs, which affects AgNPs potential, plus it produces low amounts of AgNPs [30]. In addition, electrochemical synthesis is also gaining space and is characterized by the formation of a reduced intermediate metal salt at the cathode in the presence of a stabilizing agent using an electronic device containing electrolytic cells with silver plate electrodes [31].

Among all methods to obtain AgNPs, biosynthesis is the most economical and ecologically viable alternative, as it increases stability and avoids the use of organic solvents and toxic reagents [32]. Biosynthesis is a less harmful method, and a relevant practice in the field of nanotechnology. They can be produced enzymatically and non-enzymatically, under pressure and at room temperature, without using external stabilizing agents. The reducing and stabilizing agents used in this type of synthesis are molecules produced by proteins, carbohydrates, plants, algae, bacteria, yeasts, and fungi [33, 34].

The use of plants for the synthesis of AgNPs is one of the most viable methods, as it is considered faster compared to other routes, reliable, non-toxic, and ecologically correct [35–37]. The synthesis of nanoparticles by methodologies that use biological routes has been extensively researched. The first microorganism, reported in the literature, used for this purpose was *Pseudomonas stutzeri*, and later actinomycetes, fungi, cyanobacteria, and other plant materials such as fruits, peels, and roots [36, 37].

Antimicrobial mechanism of action of AgNPs

In the last two decades, the number of bacterial infections caused by multidrug-resistant pathogenic microorganisms (MDR) has increased sharply, mainly due to the indiscriminate use of antimicrobials in clinical practice and in agriculture. Thus, there is a need for the development of new therapies that act on MDR stains, in which AgNPs have been gaining prominence [38]. The antibacterial activity attributed to AgNPs can be explained by the large surface area of nanoparticles, which allows greater contact with the microorganism, causing its death even in low concentrations [39, 40].

AgNPs adhere to the cytoplasmic membrane and cell wall of microorganisms, penetrating them and, consequently, affecting vital cell function, since they interact with cellular structures and biomolecules, such as ribosomes, mitochondria, proteins, lipids, and DNA, inducing the generation of reactive oxygen species (ROS) and free radicals, as well as in the modulation of microbial signal transduction pathways (Fig. 1) [39–41]. Another mechanism of antibacterial action occurs through the oxidation of AgNPs that leads to silver toxicity in bacterial cells. Toxicity depends on the presence of oxygen and is linked to the release of silver ions that are released by AgNPs when they come into contact with water [42].

Antibacterial activity of AgNPs against *Pseudomonas aeruginosa*

The use of silver as a therapeutic alternative for several diseases has been reported since ancient times. Prior to the advent of antibiotics, silver was used for antibacterial purposes in the treatment of patients who had open wounds and burns [43]. After the development of antibiotics, and later the use of nanotechnology as a carrier of bioactive products, silver started to be used in the form of nanoparticles. Although AgNPS have antibacterial activity against both Gram-negative and Gram-positive bacteria, it is reported that Gram-negative strains are more sensitive to AgNPs than Gram-positive strains, mainly due to the greater ease of crossing the cell wall [44–46].

Gram-negative bacteria have an outer layer of lipopolysaccharides and a thin layer of peptidoglycan. This layer of lipopolysaccharides is composed of lipids covalently that are linked to polysaccharides, having a negative charge, electrostatically attracting the positive charge of AgNPs, facilitating the adhesion of AgNPs. In the Gram-positive, the interaction of AgNPs occurs, but due to the thick layer of peptidoglycan on the cell wall, AgNPs are stationary, impairing the release and uptake of proteins, ions, sugars, among others, essential for vital cellular activities, such as the production of energy. However, regardless of the composition of the bacterial cell wall, the penetration of AgNPs will occur, and it will act against Gram-positive and Gram-negative [41, 47].

The different toxicity profiles of AgNPs against Gram-positive and Gram-negative bacteria are also observed in other microorganisms. In the case of viruses, such as HIV-1 and hepatitis B, AgNPs can inactivate viruses by inhibiting their binding to host cells, interacting with glycoproteins and thus blocking the viral entry phase, besides, in other studies, it is believed that AgNPs also cause inhibition of replication phases [48]. In bacteria, it is suggested that toxicity depends on the constitution of the microorganism's cell wall and, as observed in fungi, it interferes both in the metabolism with the generation of ROS, and through interactions with the membrane constituents that lead to cell lysis [49, 50]. Toxicity to more complex organisms, such as human cells, is possibly different due to structural and physiological differences, as well as defense mechanisms that allow exposure to high concentrations of AgNPs [48].

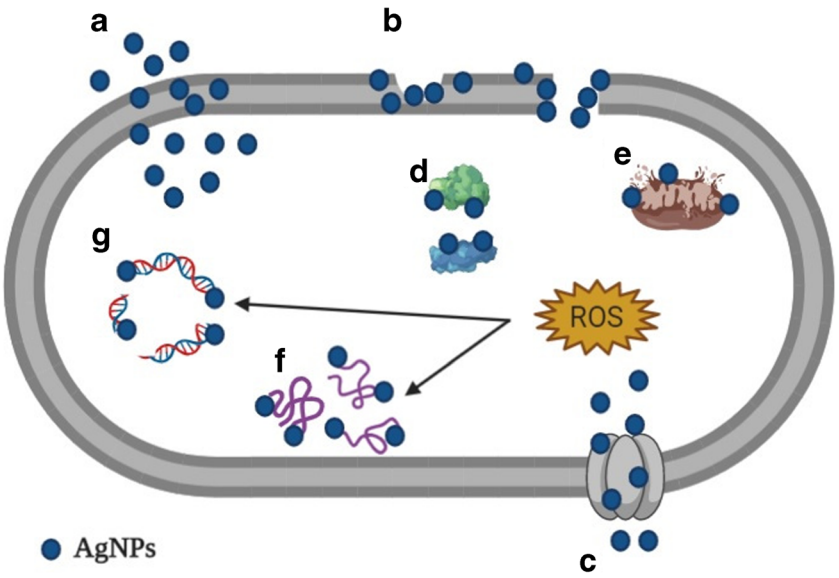

Fig. 1 (A) AgNPs attach to cell wall and penetrate membrane; (B) AgNPs damage the cell wall and membrane; (C) Entry of AgNPs by porin proteins; (D) AgNPs cause ribosome disassembly; (E) AgNPs cause mitochondrial dysfunction; (F) AgNPs cause protein denaturation; (G) AgNPs cause DNA damage; (H) AgNPs cause ROS production and oxidative stress

Antibacterial activity against *P. aeruginosa* isolates is generally considered to be more effective in AgNPs with reduced size, because they have a surface area that allows greater interaction with bacterial cells, and therefore have a promising antibacterial activity compared to larger size (Table 1). In addition, the bacterial cell membrane is negatively charged, and AgNPs are positively charged, causing them to accumulate in the membrane, causing structural changes, and making it more permeable [37].

As we can see in Table 1, the size of the AgNPs directly influences the activity of the nanoparticles, because smaller sizes increase the surface contact area of AgNPs with microorganisms, specifically *P. aeruginosa*. Arokiyaraj et al. [64] analyzed the activity of AgNPs with a size of 121 nm, obtaining MIC results of 15 μg/mL against *P. aeruginosa* strains, while Shah et al. [70] using nanoparticles with a size of 48 nm obtained MIC value of 12.5 μg/mL. Singh et al. [69] developed AgNPs with particle size of 20 and 40 nm, observing differences in activity against *P. aeruginosa* strains with MIC values of 6.25 and 12.5 μg/mL, respectively.

Values less than MIC were observed in the studies by Yuan, Peng, and Gurunathan [65] and Liao et al. [11] who obtained AgNPs with sizes of 11 and 5 nm, respectively, and tested their activity in *P. aeruginosa* strains. Yuan, Peng, and Gurunathan [65] obtained MIC value of 1 μg/mL, while Liao et al. [11] obtained an MIC value of 1.406 μg/mL.

Jasuja et al. [56] used extracts from the bark of *Punica granatum L.* (Lythraceae), to synthesize AgNPs. The results showed that the MIC was 45 μg/mL against *P. aeruginosa* ATCC, and the mechanism of action of AgNPs was possibly due to the decrease of the stiffness of the cell wall polysaccharides, inactivate the transport of enzymes, which in turn generated H_20_2 resulting in bacterial death. The same mechanism of action is reported by Arokiyaraj et al. [64] who used as source of AgNPs the root of the plant *Rheum palmatum L.* (Polygonaceae), reaching an inhibition halo of 13 mm and MIC of 15 μg/mL against *P. aeruginosa* ATCC 27853.

Singh et al. [58] used twenty MDR *P. aeruginosa* strains isolated from burned patients to investigate the antibacterial activity of AgNPs synthetized by the bark of the plant *Tinospora cordifolia* (Thunb.) Miers (Menispermaceae). The evaluation of the antibacterial activity of these AgNPs at a concentration of 12.5 to 200 μg/mL of Ag^+ by the diffusion disc method showed an inhibition zone of 10 ± 0.58 to 21 ± 0.25 mm, and MIC from 6.25 to 200 μg/mL. Another study developed by Singh et al. [60] produced AgNPs using the aqueous extract of the plant *Phyllanthus amarus* Schum. & Thonn (Phyllanthaceae). The antibacterial activity of these AgNPs was evaluated against fifteen MDR *P. aeruginosa* strains also isolated from patients who suffered burns. Using the same methods, the results of the inhibition zone in this study ranged from 10 ± 0.53 to 21 ± 0.11 mm in concentrations at 12.5 to 100 μg/mL and MIC from 6.25 to 12.5 μg/mL, where, according to the authors, MIC values are equivalent to those of standard antibiotics.

Through these results, it was possible to describe that the main mechanism considered for the release of Ag^+ from AgNPs may occur due to the phytochemical composition of plants, where terpenoids, organic acids, and flavonoids are the main mediators of reduction. In addition, both extracts also exhibit therapeutic potential against MDR *P. aeruginosa* strains, and can act in synergism with Ag^+ as therapeutic agents against bacterial infections [58].

Singh et al. [69] explored the synthesis of AgNPs from the aqueous extract of the plant *Cannabis sativa* L. (Cannabaceae). These AgNPs exhibit MIC of 6.25 μg/mL and MBC of 12.5 μg/mL against PA01 (chloramphenicol-resistant *P. aeruginosa*). Due to the small size of the AgNPs (20–

Table 1 Antibacterial activity of AgNPs against *Pseudomonas aeruginosa*

Silver nanoparticles size	Silver nanoparticles shape	Type of synthesis	*Pseudomonas aeruginosa* strains	Antibacterial activity	References
30 nm	Spherical	Chemical	NS	MIC = 2 μg/mL	[51]
22 nm	Spherical	Biosynthesis - *Tribulus terrestris*	MDR	Zone of inhibition = 9.25 mm	[52]
20 nm	Spherical	Chemical	MDR	MIC = 100 μg/mL MBC = 200 μg/mL	[53]
20 nm	Spherical	Biosynthesis - *Solanum tricobatum*	NS	Zone of inhibition = 12 mm	[54]
47 nm	NS	Chemical	PA01	10 μg/mL caused a − 5 log reduction.	[55]
15 nm	NS	Biosynthesis - *Punica granatum*	ATCC	MIC = 45 μg/mL	[56]
NE	NS	Chemical	Susceptible; MDR; ATCC 27853	Inhibition rate = 67%	[57]
36 ± 9 nm	Spherical	Biosynthesis - *Tinospora cordifolia*	Clinical isolate	MIC = 6.25–200 μg/mL	[58]
NS	NS	NS	ATCC 10145	MIC = 1 mg/mL	[59]
15.7, 24 ± 8 nm	Spherical	Biosynthesis - *Phyllanthus amarus*	Clinical isolate	MIC = 6.25–12.5 μg/mL	[60]
7 nm	Spherical	Chemical	MDR	MIC = 11.25 μg/mL	[61]
20 nm	Spherical	Biosynthesis - *Justicia adhatoda*	MTCC 741	Inhibition zone = 8–10 mm	[62]
40 nm	Spherical	Biosynthesis - *Psidium Guajava*	NS	Inhibition zone = 8–10 mm	[63]
121 nm	Hexagonal and Spherical	Biosynthesis - *Rheum palmatum*	ATCC 27853	MIC = 15 μg/mL	[64]
11 nm	Spherical	Chemical	MDR	MIC = 1 μg/mL	[65]
10 nm	NS	NS	INCQS 0230; ATCC 27853; PA01 PA02	Bacterial reduction at a concentration of 1.25 and 0.156 μg/mL	[66]
12 nm	Spherical	Biosynthesis - *Azadirachta indica*	NS	Inhibition zone = 6 mm	[67]
26.95 nm	Spherical	Biosynthesis - *Punica granatum*	ATCC 27584	Inhibition zone = 10 mm	[68]
20–40 nm	Spherical	Biosynthesis - *Cannabis sativa*	PA01 susceptible	MIC = 6.25 μg/mL; MBC = 12.5 μg/mL	[69]
5–20 nm	Spherical	Chemical	MDR	MIC = 1.406–5.625 μg/mL MBC = 2.813–5.625 μg/mL.	[11]
14–48 nm	Spherical	Biosynthesis - *Piper betle*	PA01	MIC = 12.5 μg/mL	[70]
20 ± 3 nm	Spherical	Chemical	PA14	MIC = 10 μg/mL; MBC = 20 μg/mL	[71]

NS, not specified; *MDR*, multidrug-resistant; *MIC*, minimum inhibitory concentration; *MBC*, minimum bactericidal concentration; *PA*, *Pseudomonas aeruginosa; ATCC*, American Type Culture Collection; *MTCC*, Microbial Type Culture Collection; *INCQS*, National Institute of Quality Control in Health

40 nm), they easily entered the bacterial intracellular environment, which, in turn, managed to cause oxidation of cellular components through the generation of ROS. Shah et al. [70] used AgNPs synthetized from *Piper betle L.* (Piperaceae) against the isolate PA01. The MIC was 12.5 μg/mL, and, according to the authors, it was due to the antibacterial nature of silver ions.

In the study conducted by Kora and Arunachalam [51], the AgNPs were synthesized in a solution containing 1 mM of silver nitrate with 1.6 mM of sodium dodecyl sulfate and 0.85 M of ethanol, obtaining nanoparticles with 30 nm in spherical format, highly stable and with a double layer of sodium dodecyl sulfate on its surface that demonstrated antimicrobial activity against antibiotic-sensitive *P. aeruginosa* (MIC = 2 μg/mL). However, Amirulhusni et al. [53] produced AgNPs by chemical synthesis and obtained nanoparticles with 20 nm in spherical format that exhibit antibacterial activity against ten MDR *P. aeruginosa* strains (MIC and MBC = 100 μg/mL and 200 μg/mL, respectively).

During the research performed by Yuan, Peng, and Gurunathan [65], AgNPs were produced by the chemical synthesis using quercetin, a flavonoid with five hydroxyl. These spherical AgNPs exhibit an average size of 11 nm and MIC of 1 μg/mL in cultures with MDR *P. aeruginosa*, isolated from goat's milk, unlike the results found in the study of Liao et al. [11] who obtained MIC of 2.596 ± 1.126 μg/mL and MBC of 3.246 ± 1.056 μg/mL using AgNPs synthesized chemically with 5–20 nm in spherical format against MDR clinical

isolates of *P. aeruginosa*. Silva et al. [71] performed chemical synthesis of AgNPs covered with sodium citrate. The nanoparticles exhibit size of 20 ± 3 nm and spherical format. According to the authors, light excites the local plasmonic resonance of the surface in AgNPs. From that, they verified the activity of AgNPs in bright and dark light. AgNPs present MIC of 10 μg/mL and MBC of 20 μg/mL in dark light and MIC of 5 μg/mL and MBC of 10 μg/mL in bright light.

The synergistic effect between antimicrobial drugs and AgNPs has already been tested, and it was proved that it potentiates antimicrobial action against antibiotic-sensitive and MDR *P. aeruginosa* strains. Studies have associated AgNPs with chloramphenicol, kanamycin, vancomycin, ciprofloxacin, and polymyxin B. In this sense, AgNPs optimize and facilitate the infiltration of antibiotics, allowing maximum efficiency, as well as promoting damage to the microorganism [72–74].

In the work of Ghosh et al. [72], numerous antibiotics were tested against *P. aeruginosa*, but chloramphenicol, kanamycin, and vancomycin showed better results when tested together with AgNPs. AgNPs mechanism of action is not fully elucidated in the literature, but it is known that they have a selective approach towards the cell membrane, thus destabilizing it. Chloramphenicol acts on the bacterial 50S ribosomal subunit, binding to it, thereby prevents transfer of amino acids and peptides formation. Therefore, AgNps could facilitate chloramphenicol diffusion in the bacteria. Kanamycin is an aminoglycoside and it also interferes in protein formation, binding to bacterial 30S ribosomal subunit. So, the mechanism of this synergistic antibacterial effect could be the same as chloramphenicol and AgNps together. Vancomycin inhibits cell wall synthesis, preventing further elongation of the peptidoglycan matrix, so together with AgNPs, the damage to the cell wall could increase, resulting in better antibacterial results [72, 75–78].

The association of polymyxin B with AgNPs has shown to be more effective than either of these agents alone. Jasim et al. [73] reported that this combination results in greater increase of ROS production and greater morphological changes in the outer membrane, leading to cytosolic green fluorescent protein (GFP) release. Polymyxins mechanism of action is based on the interaction of this drug with the lipid A component in the lipopolysaccharide of the outer membrane of Gram-negative bacteria; thus, AgNPs can also interact with the outer membrane, causing disruption and leading to ROS production. So, the antibacterial synergy of this combination may involve their combined membrane disruption activity and their respiratory chain poisoning activity [73].

In this sense, the choice of the antibiotic according to its therapeutic target is important when wants to associate its effects with those of AgNPs. By destabilizing the membrane, AgNPs can facilitate the entry of antibiotics whose target is intracellular and thereby facilitate its therapeutic effect. Thus, the association of AgNPs with drugs that have an intracellular target is more interesting, as it could provide synergistic effects, than drugs that have an extracellular target [78]. Brasil et al. [79] used surface-enhanced Raman scattering spectroscopy (SERS) to assess the association of AgNPs, chitosan, and the antibiotics azithromycin, levofloxacin, or tetracycline against Gram-negative and Gram-positive bacteria strains, noting that the combination promoted a reduction of 37–97% in the minimum inhibitory concentration of drugs. Deng et al. [80] observed the interaction of AgNPs with the antibiotics enoxacin, neomycin and tetracycline, showing through Raman's technique that they form complex with AgNPs and may exhibit a synergistic effect against microorganisms.

Antibiofilm mechanism of action of AgNPs

Biofilms are communities of microorganisms connected to a surface or to other microorganisms, forming aggregates wrapped in an extracellular matrix of polysaccharides, proteins, and glycoproteins. Hence, a favorable environment is formed, and the biofilm is established to act as a protector against exogenous stress [81, 82]. Biofilms may increase risks of infection, prolong hospitalizations, and raise the costs for health services, since there is a great difficulty to eradicate them. The mechanisms that are related to defense and survival of the biofilm include resistance to antibiotic therapy and gene exchanges between microorganisms, which help the pathogen to avoid host immune responses, thereby establishing chronic infections [83, 84].

In the attempt to overcome bacterial resistance, researchers are looking for new antibiotic alternatives that not only prevent the development of resistance, but also reduce the use of conventional antibiotics allowing the effective destruction of biofilms [85]. Among them, the use of AgNPs has gained a lot of attention due to its antibacterial and antibiofilm activity, since breaking through the biofilm barrier is a challenge. Thus, new strategies using AgNPs were developed in order to interrupt biofilm growth or to degrade it [47, 86, 87].

Some studies suggest that the main mechanism of biofilm destruction occurs through the binding of AgNPs in the exopolysaccharide matrix, disrupting the biofilm structure by recognizing the peptidoglycan structure present in bacterial membranes, causing physical damage, ion release, ROS production, leading to oxidative stress, and DNA damage (Fig. 2) [88, 89]. When bacteria is treated with AgNPs, morphological changes are revealed in biofilm's architecture, such as uneven cell surface, suggesting cell lysis [70], relevant morphological damages in the cell wall, membrane corrugation damage, changes in membrane polarization and/or permeability [90], and distinct EPS-matrix formation surrounding the bacterial strains [91]. Moreover, electrostatic interactions between AgNPs and bacterial membranes cause them to rupture, so that AgNPs can penetrate into the mature biofilm [86, 92].

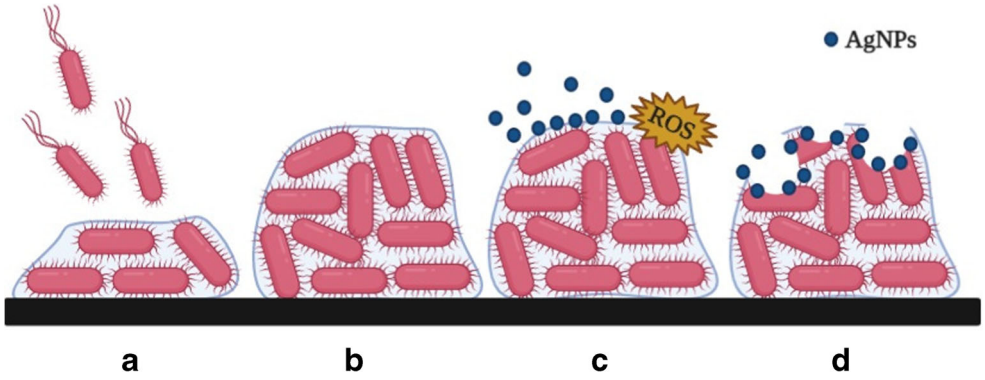

Fig. 2 (A) Attachment of cells to form biofilm; (B) Mature biofilm; (C) AgNPs bind to the exopolysaccharide matrix of biofilm and cause ROS production; (D) disruption of biofilm

Antibiofilm activity of AgNPs against *Pseudomonas aeruginosa*

The AgNPs have demonstrated biological activity against several pathogens. Thus, their actions were tested against different biofilm-producing microorganisms. Among numerous published works, many evaluate AgNPs' activity against biofilm produced by *P. aeruginosa* (Table 2).

In the work of Palanisamy et al. [57], 20 μg/mL of AgNPs inhibited the growth of biofilm from a sensitive strain with an inhibition rate of 67%. However, AgNPs inhibited the formation of biofilm of the MDR strain with an inhibition rate of 56%, indicating that MDR strains need a higher concentration of AgNPs to inhibit biofilm growth. Ansari et al. [61] tested AgNPs' antibiofilm activity against extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) producing *P. aeruginosa* strains, and biofilm formation was ceased when the biofilm was exposed to 60 μg/mL of AgNPs, but when AgNPs were tested against *P. aeruginosa* PA01 strain's biofilm, only 10 μg/mL was necessary to decrease biofilm growth significantly [94], showing once more that MDR strains need a higher concentration of AgNPs to get inhibited.

The type of synthesis can also interfere in AgNPs antibiofilm potential, especially since it can affect AgNPs' size and surface area [100]. Loo et al. [93] tested AgNPs with distinct sizes against *P. aeruginosa* PA01 biofilm and obtained different results using the same amount of AgNPs. They used AgNPs at the sizes of 8 nm, 20 nm, and 35 nm, and the 8 nm AgNPs showed better results in biofilm detachment in all different concentrations, especially due to its bigger surface area to volume which translates to a higher availability of surface area for oxidation and consequently, silver ions release, once AgNPs are exposed to liquids. In the work of Radzig et al. [94], they tested AgNPs at the size of 8.3 ± 1.9 nm against *P. aeruginosa* PA01 biofilm and a concentration of 10 μg/mL was enough to decrease biofilm growth significantly. However, when Habibipour, Moradi-Haghgou and Farmany [99] tested AgNPs with size ranged of 32–85 nm against *P. aeruginosa* PA01 biofilm, concentrations between 100 and 500 μg/mL were necessary to inhibit biofilm growth significantly. Showing once more that smaller AgNps have better activity, and demand a smaller concentration of AgNPs in the treatment.

AgNPs may be used against biofilm as pre-treatment to inhibit biofilm formation or post-treatment to reduce biomass and destroy biofilm formed by *P. aeruginosa*. In pre-treatment, it can inhibit biofilm growth in a dose-dependent manner, as Shah et al. [70] and Habibipour, Moradi-Haghgou, and Farmany [99] indicated. In the studies of Shah et al. [70], they reported that AgNPs synthesized from *Piper betle*, inhibited 14.33 ± 4.6% of *P. aeruginosa* PA01 biofilm formation at a concentration of 2 μg/mL, 36.10 ± 5.4% at a concentration of 4 μg/mL, 55.09 ± 2.62% at a concentration of 6 μg/mL, and 78.20 ± 3.1% at a concentration of 8 μg/mL. Habibipour, Moradi-Haghgou, and Farmany [99] tested different concentrations of AgNPs (0.05 mg/mL to 0.5 mg/mL), and when they using concentrations that were higher than 0.1 mg/mL, the AgNPs were able to decrease the biofilm formation.

Used as post-treatment therapy, AgNPs were able to reduce *P. aeruginosa* CCM 3955 biofilm viability by 46.28%, 65.50%, and 92.43% and reduce biomass by 5.69%, 37.87%, and 67.52%, after treatment with concentrations at 2, 6, and 12 μg/mL, respectively [91]. Singh et al. [69] evaluated the antibiofilm activity of AgNPs produced from *Cannabis sativa* against *P. aeruginosa* PA01 and as a result showed a decrease in biofilm's viability at a concentration at 50 μg/mL. Pompilio et al. [90] tested AgNPs synthesized electrochemically and also showed a reduced biofilm's viability, achieving biofilm erradication at a concentration of 17 μg/mL.

Furthermore, AgNPs can also work as an enhancer of antimicrobials action against biofilm. AgNPs work in a synergic form with tobramycin against *P. aeruginosa* PA01 biofilm, causing extensive cellular changes, including altered cellular morphology and cytoplasmic clearing, and eliminating biofilm formed by this strain [14]. AgNPs combined with polymyxin B has also presented an increase in its antibiofilm activity against antibiotic-sensitive and MDR *P. aeruginosa* clinical isolates even in low concentrations, when compared to its action alone [101]. Aztreonam can also work in

Table 2 Antibiofilm activity of AgNPs against *Pseudomonas aeruginosa*

Silver nanoparticles size	Silver nanoparticles shape	Type of synthesis	*Pseudomonas aeruginosa* strains	Antibiofilm activity	References
30 nm	Spherical	Chemical	NS	1 μg/mL of AgNPs inhibited biofilm formation by 95 ± 0.62%.	[51]
47 nm	NS	Chemical	PA01	10 μg/mL of AgNPs caused a 3 log inactivation of biofilm cells.	[55]
7–70 nm	Spherical, pseudo-spherical and a few with a cylindrical shape.	Chemical	PA01	600 μg/mL of AgNPs (8 nm) resulted in approximately 90% of biofilm detachment.	[93]
7 nm	Spherical	Chemical	ESBL, MBL and NON-ESBL clinical isolates	60 μg/mL of AgNPs completely blocked biofilm formation.	[61]
8.3 ± 1.9 nm	Spherical	Biosynthesis	PA01	A decrease of the bacterial mass in the biofilm was seen when ~ 10 μg/mL of AgNPs was used.	[94]
NS	NS	Chemical	ATCC 27853; Susceptible and MDR clinical isolates.	20 μg/mL of AgNPs inhibited the growth of biofilm from the sensitive strain in 67% and from the MDR strain in 56%.	[57]
2–10 nm	Spherical	Biosynthesis - *Allophylus cobbe*	NS	0.5 μg/mL of AgNPs decreased biofilm activity by more than 90%.	[95]
14 nm	Predominantly spherical, but a few had an oval shape	Biosynthesis - *Lagerstroemia speciosa*	Clinical isolate	50 μg/mL of AgNPs inhibited 86.73 ± 28%.	[96]
20–40 nm	Spherical	Biosynthesis - *Cannabis sativa*	PA01	50 μg/mL of AgNPs inhibited more than 80% of biofilm formation.	[69]
15–30 nm	Spherical	Biosynthesis - *Rhodiola rosea*	PA01	50 μg/mL of AgNPs inhibited more than 80% of biofilm formation.	[97]
55.6 ± 2.9 nm	Quasi-spherical	Electrochemical	DIN1	17 μg/mL of AgNPs reduced biofilm viability in more than 90%.	[90]
10–15 nm	Spherical	Biosynthesisl -*Nardostachys jatamansi*	NS	64 μM of AgNPs prevented biofilm formation.	[98]
14–48 nm	Spherical	Biosynthesis - *Piper betle*	PA01	8 μg/mL of AgNPs reduced biofilm formation in 78.20 ± 3.1%.	[70]
31.49 ± 2.48 nm	Spherical	Chemical	CCM 3955	18 μg/mL of AgNPs completely inhibited biofilm growth.	[91]
32–85 nm	Spherical	Biosynthesis - Black peel pomegranate	ATCC 10662	100 to 500 μg/mL of AgNPs inhibited biofilm formation significantly.	[99]

NS, not specified; *PA*, *Pseudomonas aeruginosa*; *MDR*, multidrug-resistant; *ESBL*, extended-spectrum β-lactamase; *MBL*, metallo-β-lactamase; *ATCC*, American Type Culture Collection; *CCM*, Czech Collection of Microorganisms

synergism with AgNPs, reducing *P. aeruginosa* PA01 biofilm biomass and viability in a dose-dependent manner, as well as reducing biofilm thickness and causing cellular [102]. Ampicillin was also tested together with AgNPs and the results were about three times more effective comparing to AgNPs antibiofilm action alone [95].

Conclusions

AgNPs are rapidly obtained through green synthesis with the use of plants and/or microorganisms without the development of toxic waste to the handler and the environment. More and more studies show the antimicrobial activity of AgNPs and their importance in the insertion of antibacterial therapy. AgNPs exhibit potential against gram-negative bacteria, with antimicrobial activity and promising antibiofilm activities against *Pseudomonas aeruginosa* resistance profile multidrug-resistant strains, due to the small concentrations capable of promoting rapid cytotoxicity in the microorganism and, consequently, death. It is worth mentioning that the size of the obtained AgNPs is important because the surface area of contact with the microorganism is greater in smaller nanoparticles (20–40 nm), thereby enhancing its antimicrobial effect. In vivo studies should be developed to better assess the safety of administering AgNPs.

Code availability Not applicable.
Data availability Not applicable.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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