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# Biosynthesized Silver Nanoparticles as Promising Antimicrobial Agents and Their Mode of Action

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# Abstract

The rise of multidrug-resistant bacteria among pathogenic microorganisms poses a significant challenge and has prompted the exploration of alternative antibacterial agents. Nanoparticles, particularly metals, have gained attention as potential alternatives to traditional antimicrobials in recent years. Silver nanoparticles (Ag NPs) possess strong antimicrobial properties, attracting considerable attention. This renders them favorable for utilization across various applications. Despite displaying promising antibacterial properties, the precise mode of action remains under investigation. This review integrates past reviews and recent research aimed to outline the mechanisms behind the antimicrobial activity of Ag NPs. Key areas of discussion encompass the synthesis of Ag NPs, especially the green synthesis, the correlation between Ag NPs and ions and the antimicrobial effect of Ag NPs. The interaction with vital cell parts like cell wall, cell membrane, DNA, and cellular enzymes also the generation of ROS, considering factors such as physicochemical properties of Ag NPs that influence their efficacy are also included. Furthermore, the review also addresses the potential development of resistance by pathogenic microbes. The potent antimicrobial properties of Ag NPs, making them valuable across diverse applications such as medical, pharmaceutical and consumer products. Future applications hold promise in nanomedicine, agriculture, food industry, and environmental protection fields.

Keywords: Silver nanoparticles, antimicrobial agent, ROS, biosynthesis, mode of actions

# 1. Introduction:

The advent of nanotechnology in the 21<sup>st</sup> century is seen as the onset of a new phase in technological advancement. Metal nanoparticles (NPs) have garnered significant attention in recent years due to their unique optical and physicochemical characteristics, rendering them versatile for various applications across different fields of study [1]. The emergence of microbial resistance to both antibiotics and metal ions has shifted the focus of many researchers to metal NPs. Through their unique properties, NPs offer a potential solution

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doi 10.21608/aels.2024.291232.1057

to these resistance challenges [1]. Metal NPs have nano size, large surface area, and distinct physical and chemical properties that enable them to interact effectively with biological molecules and systems [2]. Silver nanoparticles (Ag NPs) are highly regarded for their antimicrobial characteristics and are employed in many applications. They are recognized for their potential as anti-fungal, antibacterial, and antiviral agents, thus Ag NPs are currently being proposed as an alternative to silver ions and antibiotics [3], [4]. This review aims to investigate the biosynthesis, antimicrobial mechanisms, and efficacy of biosynthesized Ag NPs, emphasizing their potential as a sustainable and effective approach to combating the growing issue of antimicrobial resistance.

#### 2. The Challenge Posed by Antibiotic Resistance:

One of the notable advancements in recent decades has been the emergence of antibiotics. During the mid-20<sup>th</sup> century, antibiotics were hailed as miraculous treatments because of their effectiveness in combating diverse infections and diseases [5], [6]. During the period from 1930 to 1970, antibiotics effectively combat infectious microorganisms and save countless lives [7], [8]. Later, their reputation has somewhat declined due to overuse and misuse, leading to the emergence of antimicrobial resistance (AMR) which is recognized as a global health concern [6], [9]. Microorganisms adapted to antibiotics are also known as multidrug-resistant (MDR) organisms. Bacteria are adapting to antibiotics by various means, including mutations in genes associated with antibiotic mechanisms or acquiring foreign DNA encoding resistance factors [5], [10]. Considering this, it has become essential to seek alternatives to conventional antibiotics.

#### 3. Nanoparticles (NPs:

Nanotechnology is a modern scientific discipline that involves the manipulation and utilization of tiny materials referred to as nanomaterials, typically sized at no more than 100 nm in at least one dimension. These materials typically exhibit unique size-dependent properties distinct from those of bulk materials [11]. Nanoparticles, fundamental to nanotechnology, are particles characterized by their dimensions being within the nano-scale range in all external aspects [12]. The physical and chemical attributes of bulk substances remain constant, whereas NPs exhibit varying physical properties based on their size. For instance, they possess a larger surface area compared to bulk materials due to the smaller particle size [13]. Furthermore, NPs display distinctive optical characteristics such as color, light penetration, absorption, and reflection properties [12]. Utilizing NPs as part of an innovative antimicrobial approach represents a potential strategy to combat the emergence of MDR organisms [7]. The use of bimetallic nanoparticle materials holds promise for contributing to several Sustainable Development Goals (SDGs): SDG 3 (Good Health and Well-Being), SDG 6 (Clean Water and Sanitation), SDG 12 (Responsible Consumption and Production), and SDG 9 (Industry, Innovation, and Infrastructure). The progress in nanotechnology, particularly the creation of Ag NPs, aids in achieving SDG 9 by enhancing resilient infrastructure, encouraging sustainable and inclusive industrialization, and promoting innovation [**53**].

# 4. Synthesis of Nanoparticles:

The production of NPs encompasses physical, chemical, and biological methods [14], [15]. These techniques can be classified as either top-down or bottom-up approaches [12]. In the top-down approach, the systematic division of a system into nano-sized components via a range of physical and chemical methods is conducted [16]- [17]. Utilizing techniques such as laser, thermal, and mechanical milling facilitates this process [18]. Although top-down techniques are straightforward to implement, they prove inadequate for producing extremely small and irregularly shaped particles [17], [19]. The term "constructive technique" characterizes the bottom-up approach, contrasting markedly with the top-down method. In the bottom-up approach, atoms and molecules act as the foundational elements for nanomaterials, self-assembling to form NMs with precise shape, size, and chemical composition [19], [55]. physical methods for NPs creation require substantial costs [20], and chemical synthesis involves posing environmental hazards and yielding toxic byproducts [15]. Unlike the biosynthesis of NPs which used to benefit from the reductive properties of proteins and metabolites present in various biological systems, such as plants, algae, and microorganisms. Biosynthesis offers an eco-friendly and non-toxic means, so it's considered the best method [17].

# 5. Biosynthesized Silver Nanoparticles (Ag NPs :

Silver NPs possess attractive biological, physical, and chemical attributes, finding extensive use in

various biological applications owing to their antimicrobial properties [22]- [23]. Microorganisms such as bacteria, fungi, and yeast are utilized in the biosynthetic process. Biosynthesis proves environmentally benign, economical, and minimizes waste [24], [32]. When using a microbe to synthesize NPs, microbes' defense mechanisms facilitate nanoparticle creation. Microbial resistance mechanisms against heavy, toxic metals are the interaction between cells and metals, converting soluble metals into insoluble, non-toxic NPs [14], [21] . Occasionally, Ag NPs synthesis by microorganisms can occur through various processes, including biosorption or intracellular bioaccumulation. Bacteria are prominently featured in the production of metal NPs, particularly silver. Synthesis of Ag NPs through bacterial action can occur via two routes: extracellular and intracellular. In intracellular synthesis, silver ions attracted by the negatively charged bacterial cell wall, enter the cell, and are subsequently reduced by bacterial enzymes to form Ag NPs. Conversely, in extracellular synthesis, reducing agents present in microbial secretions are collected and utilized, with enzymes like nitrate reductase playing a crucial role in bio-reduction reactions [21], [25].

## 5.1. Synthesis of Ag NPs using plants

Numerous plants have been reported to synthesize nanoparticles. Various parts of plants, such as leaves, roots, bark, peel, stems, and seeds, are utilized in the synthesis process [78]. The seasonal nature of plants and the influence of environmental conditions, which vary by region, can affect their chemical composition, and potentially hinder their use in nanoparticle synthesis [74]. Plants contain a variety of biomolecules that serve as reducing and stabilizing agents in the biosynthesis process [85], [88] .The synthesis of Ag NPs was documented using leaf extract from Sida cardifolia [67]. Similarly, another study detailed the production of Ag NPs in various sizes employing leaf extracts from Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica, and peel extract from Citrus sinensis combined with a silver nitrate solution [79].

#### 5.2. Algae-Based Ag NPs Synthesis

Numerous studies in the literature highlight algae as efficient "nano factories" for environmentally friendly synthesis of Ag NPs [**70**]. For instance, Chaetomorpha linum facilitates the formation of Ag NPs through the reduction of silver metal ions [**75**]. Additionally, the seaweed extract from the marine algae Caulerpa racemosa has been utilized for the synthesis of Ag NPs, yielding particles in the size range of 5-25 nm [**76**].

#### 5.3. Synthesis of Ag NPs via microorganisms

Utilizing microorganisms for nanoparticle production is regarded as a safe, environmentally friendly, and cost-effective approach [83]. Various microorganisms, including fungi, yeast, bacteria, and actinomycetes, have emerged as promising candidates for nanoparticle fabrication [82].

#### 5.3.1. Synthesis of Ag NPs via Fungi

Fungi have garnered significant attention for nanoparticle synthesis [76]. For instance, Fusarium oxysporum has been noted for its extracellular reduction of silver ions in aqueous solutions, resulting in the formation of Ag NPs ranging from 20 to 50 nm [69]. Moreover, the filamentous fungus Neurospora crassa serves as a potent "nano factory," producing metallic gold nanoparticles (Au NPs) and Ag NPs [68]. Saccharomyces cerevisae has been extensively studied for its role in synthesizing Ag NPs and Au NPs, alongside Rhodotorula glutinis and Geotrichum candidum, which have demonstrated the ability to synthesize Ag NPs of various sizes and shapes [77].

#### 5.3.2. Synthesis of Ag NPs via Bacteria

Bacteria exhibit remarkable potential for the synthesis of metal nanoparticles due to their ease of handling and rapid growth rate [83]. Bacteria, capable of thriving in harsh and toxic environments with high concentrations of heavy metals, have evolved natural defense mechanisms to combat such conditions [58], [73]. These protective mechanisms enable bacteria to synthesize metal nanoparticles both extracellularly and intracellularly [25].

The initial evidence of bacteria's capability for synthesizing Ag NPs was demonstrated using Pseudomonas stutzeri AG259 strain, which was isolated from a silver mine [87]. Numerous bacterial species have been documented for their ability to synthesize metal nanoparticles, including Bacillus sp., Escherichia coli, Klebsiella sp., and Acetobacter sp. [71], [84]. Additionally, thirteen types of cyanobacteria were studied to assess their capacity for synthesizing Ag NPs, yielding particles ranging in size from 38 to 88 nm [72].

#### 5.3.3. Synthesis of Ag NPs via Actinomycetes

Actinomycetes derived from diverse environments have emerged as promising candidates for metal nanoparticle synthesis. Notably, Streptomyces sp. has been predominantly associated with the production of Ag NPs and Au NPs in reported studies [81]. Numerous studies highlight the efficacy of Rhodococcus spp., Streptomyces sp., Nocardiopsis sp., and Streptacidiphilus spp. as agents for manufacturing nanoparticles, encompassing the synthesis of silver, gold, and copper NPs [81], [70]. The biosynthesis of spherical Ag NPs has been documented in Streptomyces glaucus, with sizes ranging from 5 to 25 nm [89]. Recent studies have utilized cell-free extracts of Streptomyces hirsutus strain and Streptomyces thinghirensis sp. to produce spherical nanoparticles, with sizes ranging from 6 to 40 nm [80], [86].

#### 6. Ag NPs Versus Ionic Silver:

After water immersion, Ag NPs undergo an oxidation process facilitated by dissolved molecular oxygen, releasing silver ions (Ag<sup>+</sup>). Following several hours, a stable state is achieved within a specific system under certain conditions; therefore, NPs not fully dissolved by molecular oxygen [**26**]. Subsequently, under humid conditions, Ag<sup>+</sup>initiates the formation of new, smaller silver NPs in three stages (**Figure 1**). First, oxidative dissolution of Ag NPs occurs, then Ag<sup>+</sup> migrates away from the parent NPs within the layer of adsorbed water and finally a reduction of Ag<sup>+</sup> leads to the creation of new Ag NPs [**27**]- [**28**].

In contrast to bulk materials, NPs exhibit substantially greater toxicity at cellular, subcellular, and biomolecular levels [**31**]. Nano-silver has several advantages that render it an appealing option for antimicrobial treatments. Even at low concentrations, it exhibits significant efficacy against a wide range of microorganisms and parasites (with complete bacterial growth inhibition occurring at just a few  $\mu$ g/ml) [**14**]. At these levels, Ag NPs are cost-effective, easily accessible, and pose minimal systemic harm to humans [**30**].

Numerous investigations have linked the antimicrobial efficacy of Ag NPs to the liberation of Ag<sup>+</sup>. Silver ions possess the capability to bind with thiol groups (SH) in proteins, forming stable bonds. This interaction can alter the three-dimensional (3D) structure of proteins, hindering active binding sites and impeding the production of adenosine triphosphate (ATP) [23], [29]. Furthermore, various biomolecules such as DNA, RNA, and peptides readily form insoluble complexes with silver ions, inhibiting cellular division and reproduction [29]. Despite disparities among Ag<sup>+</sup>, Ag NPs, and bulk silver (Figure 2), there is a significant challenge in biological studies that take into account the effects of Ag NPs and Ag<sup>+</sup> [28]. While Ag<sup>+</sup> exhibits activity at lower concentrations, they elicit effects comparable to those induced by Ag NPs in numerous experiments. However, it has been observed that Ag NPs generate more ROS compared to silver ions, suggesting that reactive oxygen species (ROS) formation is attributable to the distinctive properties of Ag NPs rather than ions release [28]. While silver ions' impact is short-lived, Ag NPs have emerged as a solution due to their enhanced antimicrobial efficacy [4].

#### 7. Antimicrobial activity of Ag NPs:

Various studies have demonstrated the effectiveness of Ag NPs against a broad spectrum of pathogen groups [**59**], [**69**]. Ag NPs and silver ions exhibit inhibitory and lethal effects on bacterial species including E. coli and Staph. aureus, as well as yeast. Studies have found that yeast and E. coli are inhibited even at low nanoparticle concentrations, whereas Staph. aureus experiences only mild growth inhibition [**36**], [**91**]. The activity of Ag NPs has been tested on E. coli, Vibrio cholerae,



Figure 1: Stages of Ag NPs dissolution and reduction.

Ag ions	Ag NPs	Bulk material
$\begin{array}{c} & Ag^+ \\ Ag^+ & Ag^+ \\ Ag^+ & Ag^+ \end{array}$		
<ul> <li>No surface area</li> <li>Highly reactive</li> <li>Readily enters cells</li> <li>Active for short period</li> </ul>	<ul> <li>huge surface area</li> <li>extraordinarily receptive</li> <li>Potential oxidative damage</li> <li>Biomolecules binding</li> <li>Active for long period</li> </ul>	<ul> <li>&gt; little surface area</li> <li>&gt; Low capacity for oxidation</li> <li>&gt; No cellular uptake</li> </ul>

Figure 2: The main differences between bulk, nanoparticulate, and ionic silver

Pseudomonas aeruginosa, and Salmonella enterica Typhi, all of which are Gram-negative bacteria. The nanoparticles were found both in the bacterial membranes and inside the cells, demonstrating their ability to penetrate the cell membrane and enter the cell **[38].** 

Moreover, the antimicrobial activities of Ag NPs were evaluated against various pathogenic organisms. The highest sensitivity was observed against Methicillin-resistant Staphylococcus aureus (MRSA), followed by Methicillin-resistant Staphylococcus epidermidis (MRSE) and Streptococcus pyogenes. In contrast, moderate antimicrobial activity was observed against Salmonella Typhi and Klebsiella pneumoniae [**92**].

#### 8. Antimicrobial Mode of Action of Ag NPs:

While various disinfection mechanisms involving Ag NPs have been outlined, the exact mode of action remains unrevealed **[23].** Silver NPs gradually envelop microorganisms and infiltrate cells, disrupting essential processes. Due to their numerous parallel modes of action, Ag NPs hold promise as effective antimicrobial agents across various applications **[32].** The antimicrobial efficacy of Ag NPs is attributed to four distinct mechanisms: (1) adherence to cell wall and membrane surfaces; (2) intracellular penetration and disruption of intracellular structures and biomolecules such as lipids, DNA, and proteins; (3) cellular toxicity and oxidative stress induced by the generation of ROS and free radicals; and (4) alteration of signal transduction pathways **[32].** 

#### 8.1. The Effect of Ag NPs on Microbial Cell Wall:

The primary function of cell walls is to shield the cell from various environmental stresses and to determine the cell's shape [**33**]. The composition and thickness of microorganisms' cell walls influence the antimicrobial potential of Ag NPs. Studies suggest that Ag NPs penetrate bacterial cell walls, leading to significant structural alterations in the cell membrane, increased permeability, unregulated transport across the cytoplasmic membrane, and ultimately, cell death [**34**].

Research indicates that Ag NPs exhibit greater efficacy against Gram-negative bacteria compared to Gram-positive bacteria due to differences in the organization of peptidoglycan. Gram-positive bacteria have a thicker peptidoglycan layer (approximately 30 nm), whereas Gram-negative bacteria possess a thinner layer (3-4 nm). Consequently, the diffusion of Ag NPs into the cellular environment is hindered by the thickness of cell wall. Gramnegative bacteria are more susceptible to Ag NPsbased antimicrobial therapy [32], [34]. Conversely, other researchers suggest that Gram-positive bacteria are more affected by Ag NPs compared to Gram-negative bacteria [30], [35]. This finding explains that Ag NPs or the released Ag<sup>+</sup> interact with bacterial cell walls, forming "pits" due to their nano size. Subsequently, Ag NPs accumulate in these pits and form stronger connections with deeper layers [39], [35].

Upon exposure to Ag NPs, NPs adhere to bacterial cell walls and membranes based on their size and charge [**35**]. Interaction between Ag NPs and the peptidoglycan layer results in the formation of pits, N-acetylglucosamine and N-acetylmuramic acid molecules are released into the culture media, eventually leading to bacterial cell wall destruction (**Figure 3**). Furthermore, Ag<sup>+</sup> released from Ag NPs may interact with sulfur-containing proteins in bacterial cell walls, potentially causing structural damage and rupture [**30**], [**35**]. This interaction triggers noticeable morphological changes, including cytoplasm shrinkage, membrane separation, and eventual cell wall rupture [**32**], [**59**].

Research conducted on Escherichia coli (E. coli) demonstrated that a 12 nm-sized Ag NPs created pits on cell wall, destabilizing the outer membrane and leading to the escape of lipopolysaccharides (LPS) molecules and membrane proteins, ultimately resulting in cell death [36]. Additionally, it has been observed that Ag NPs (1–10 nm) attach to the cell wall of E. coli, disrupting normal functions such as permeability and respiration [**37**]- [**38**].



Figure 3: Proposed mechanism of Ag NPs on bacterial cell wall, (a) interaction, (b) pits formation and degradation of peptidoglycan.

A study explained that when exposed to Ag NPs, fungal cell walls experience overall distortion, pore formation, and surface reduction [60]. The fungal cell wall is constructed mainly from polysaccharides, chitin, and glycoproteins, which make up both the outer and inner layers of the cell wall [90]. Another research demonstrated that Ag NPs act against fungi primarily by forming insoluble compounds containing sulfhydryl groups in the fungal cell walls and by disrupting membrane-bound enzymes and lipids, leading to cell lysis [**61**].

## 8.2. The Effect of Ag NPs on The Plasma Membrane:

The interaction mechanism of Ag NPs with plasma membranes and their cellular entry remains unclear. There is a hypothesis that the initial interaction between the NPs and bacterial cells may result from the electrostatic attraction between the positively charged NPs and the negatively charged cell membranes. Nonetheless, this theory may not fully explain the attachment and uptake of negatively charged Ag NPs. It is also theorized that sulfur-containing proteins on the

membrane might be the primary interaction sites for Ag NPs, disrupting normal membrane functions [39]. Additionally, it is suggested that Ag NPs and ionic silver may alter the unsaturated fatty acids' portion. This alteration in fatty acid composition includes the isomerization from cis to trans unsaturated fatty acids that affect membrane fluidity. Such changes might modify membrane characteristics, increasing permeability and compromising membrane integrity. Consequently, treatments involving Ag NPs often result in the inflow of substances and the leakage of intracellular contents [40]. Exposure to Ag NPs was observed to induce damage to both the cell membrane and cell wall of Candida albicans (C. albicans), resulting in increased membrane permeability and the release of potassium ions [61]. Similarly, research on three bacterial species-Staphylococcus aureus (Staph. aureus), Acinetobacter baumannii, and Serratia marcescens showed that Ag NPs induce structural alterations in the cell membrane, leading to heightened permeability and eventual cell death [62].

## 8.3. Raising of ROS Levels in Cells Due to Ag NPs:

ROS are a subset of free radicals that include oxygen which are produced as byproducts of normal cellular metabolism. An increase in ROS levels within the cell leads to oxidative stress, causing cell damage through mechanisms such as lipid peroxidation, disruption of ATP synthesis, and hindrance of DNA replication [24], [32], [37]. The cell possesses mechanisms to reduce ROS concentrations, utilizing antioxidant enzymes like superoxide dismutase (SOD), catalase, and glutathione peroxidase [23], [41].

Ag NPs have been studied for their potential to induce cytotoxicity through ROS generation. Elevated ROS levels observed in cells exposed to Ag NPs suggest a significant increase in oxidative stress, leading to cell inactivation. It's suggested that Ag NPs may either catalyze ROS production or disrupt the cellular pathways regulating ROS levels [42]. Ag NPs can facilitate the electron transfer to molecular oxygen, generating superoxide radicals and initiating a cascade of radical formation or interacting with respiratory enzymes, resulting in ROS production [**37**], [**43**] . Silver ions can disrupt the cellular ROS management system, raising ROS levels inside cells. This disruption occurs directly due to Ag<sup>+</sup> strong affinity for thiols that affects antioxidant enzymes like glutathione peroxidases or indirectly through the inhibition of its synthesis pathway [23], [44]. The study conducted on Fusarium graminearum revealed a notable increase in the production of ROS in mycelia treated with Ag NPs compared to untreated mycelia [63]. Another research demonstrated a significant rise in ROS levels in cells of E. coli and Staph. aureus when treated with Ag NPs compared to untreated cells [**64**].

# 8.4. The Effect of Ag NPs on the Internal Structures:

Upon entering the cell, Ag NPs and ions engage with every element within the microbial cell, either directly or via the previously mentioned ROS [**39**], [**45**].

# 8.4.1. The Effect of Ag NPs on Enzymes and Cellular Proteins

Studies have shown that within 30 minutes of exposure, Ag<sup>+</sup> released from Ag NPs penetrates bacterial cells and attaches to proteins, nucleic acids, and cytoplasmic components [46]. Research on E. coli found that Ag<sup>+</sup> distort ribosomes and block the production of proteins and enzymes crucial for ATP generation, highlighting the interaction with the ribosome as a key bactericidal action of silver ions [47]. The action of  $Ag^+$  is also attributed to damaging membrane proteins and disrupting respiratory enzymes by increasing ROS production [58]. Exposure to Ag NPs alters the expression of envelope and heat shock proteins in E. coli cells [59]. Thiol groups (-SH) in microbial cell proteins, vital for enzymatic activities, are targeted, disrupting key cellular processes such as respiration, altering protein structures and blocking active sites [29], [48], [50]. It has been noted that the thiol group of cysteine forms -S-Ag upon reacting with Ag<sup>+</sup>, affecting the protein's enzymatic functions [51], [57]. In a study on E. coli, it was observed that exposure to Ag NPs resulted in ribosomal deformation and suppression of the expression of proteins and enzymes crucial for ATP generation [65]. Another study conducted showed that

Ag<sup>+</sup> released from Ag NPs quickly penetrates bacterial cells within 30 minutes of exposure, binding to various cellular components including proteins, nucleic acids, and cytoplasmic structures [**66**].

# 8.4.2. The Effect of Ag NPs on DNA

DNA holds the most crucial genetic information within cells, and any harm to it can lead to mutations or the death of an organism. Research on E. coli and Staph. aureus has shown that DNA condensation occurs due to Ag<sup>+</sup> released from Ag NPs entering the cells by penetrating the cell wall [50]. Further studies on E. coli revealed that Ag NPs target the exocyclic nitrogen atoms in the bases of guanine, cytosine, and adenine, leading to DNA mutations. This interaction also results in mutations in essential DNA repair genes (mut Y, mut S, mut M, mut T, and nth) and causes DNA damage, such as strand breaks [32], [40]. Silver ions are known to form complexes with nucleic acids, predominantly interacting with nucleosides. They disrupt the hydrogen bonds between base pairs of the anti-parallel DNA strands, intercalating between purine and pyrimidine bases, thereby breaking the double helical structure of DNA [32], [52].

### 9. Conclusion:

In summary, this review provides insights into key questions concerning the antimicrobial efficacy of Ag NPs. Ag NPs demonstrate wide-ranging biocidal efficacy against bacteria and fungi, driving their utilization across numerous biomedical, environmental, and consumer product applications. While the precise antibacterial mechanisms of silver nanomaterials remain incompletely understood, existing theories propose a combination of mechanisms: (1) release of silver ions, (2) generation of ROS, and (3) direct interactions between nano-sized silver and cell membranes. The ability of Ag NPs to target multiple components within microbial cells makes it challenging for the microbe to develop resistance against them. Urgent studies are needed to explore the mechanisms, prevalence, and epidemiology of bacterial resistance to silver, as this information remains insufficient. Furthermore, since Ag NPs are released from various products and can be environmentally toxic, understanding their transport behavior is crucial. It's essential to quantify and qualify silver in the environment [54].

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