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GOLD AND SILVER NANOPARTICLES: ANTIMICROBIAL QUALITIES AGAINST PATHOGENS RESISTANT TO NUMEROUS MEDICATIONS

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Abstract
The rising threat of multi-drug-resistant pathogens has made way for new alternatives in antimicrobial agents, and gold (AuNP) and silver nanoparticles (AgNP) have arisen as promising candidates because of their distinct physicochemical properties. This study seeks to review and compare the effectiveness of AuNPs and AgNPs against relevant drug-resistant human pathogens, specifically MRSA, E. coli, and Pseudomonas aeruginosa. The study measured levels of nanoparticle-induced bacterial inhibition and cellular oxidative stress through the use of MIC, MBC, agar diffusion assays, and analysis of the generation of reactive oxygen species. Results from this study show that MIC and MBC values for AgNPs were lower compared to AuNPs, and they also had larger zones of inhibition, indicating more pronounced antimicrobial activity. In addition, measurement of ROS proved the presence of increased oxidative stress in AgNPs treated bacterial cells, which thus indicates one of the probable mechanisms that may explain their higher efficacy. In general, both AuNPs and AgNPs display promising potential in acting against infections elicited by antimicrobial activity, but AgNPs were found to be more potent than AuNPs with relevance to designing future treatments against multi-drug-resistant infections. However, preliminary cytotoxicity observations in relation to assessment motivate the exercises for careful consideration of dosage by balancing efficacy and safety as proposed to address future therapeutic applications.

Keywords: *Gold nanoparticles (AuNPs), Silver nanoparticles (AgNPs), Multi-drug-resistant pathogens, antimicrobial activity, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Reactive Oxygen Species (ROS)*

1. INTRODUCTION

Globally, the rise of antibiotic-resistant microorganisms is becoming a major issue. In order to battle the bacterial resistance that kills 33,000 Europeans per year, new strategies are needed [1]. A major hazard may arise from the increase of nosocomial and acquired infections since current antibiotic therapies are inefficient against multi-drug-resistant bacterial pathogens (MDRs) [2]. Even while medicines are crucial in the fight against bacterial illnesses, many microorganisms have recently

become resistant to them. The development of antimicrobial resistance (AMR) occurs when bacteria and other microorganisms modify their DNA to resist or even flourish when antibiotics are present [3]. The abuse of antibiotics and the emergence of antibiotic resistance are causes for increasing concern worldwide. Since viral infections like influenza and SARS-CoV-2 are becoming more common in 2019, it's critical to promptly evaluate risks and begin prevention and treatment. A link between SARS-CoV-2 and AMR has been warned about recently [4,5].

Microbes that are able to resist the effects of drugs intended to eliminate them are said to have antimicrobial resistance (AMR). Drug-resistant diseases presently cause around 0.7 million fatalities annually, and by 2050, the number of deaths from antimicrobial resistance (AMR) could surpass 10 million [6]. Therefore, one of the main causes of death and a serious threat to human health is antimicrobial resistance (AMR). AMR is a complicated system with several potential causes, such as the host, particular bacterial strains, and well-established resistance mechanisms.

The AMR process includes inhibitory drug uptake, inhibitory drug inactivation, target change, and active drug efflux [7]. Furthermore, three documented ascending stages of AMR—XDR, MDR, and PDR—have been identified [8]. A lack of resistance to at least one therapy was used to define XDR in each case where two or fewer antibiotic kinds were mentioned. PDR was characterized as the inability to be susceptible to all agents in all kinds of antibiotics. Multidrug-resistant bacteria were identified as those that have become resistant to three or more distinct antibiotic classes [9, 10]. To address the issue of resistance, we will need to modify the antimicrobial application protocols such that these therapies are only administered after all other treatment options have been exhausted.

The emergence of antimicrobial drug resistance is a significant global public health concern. Numerous nosocomial severe infections are caused by the 'ESKAPE' bacteria, which include Enterococcus fecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. These bacteria are multidrug resistant (MDR) [11]. Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterobacteriaceae are among the multi-resistant bacteria that have been identified as priority one pathogens by a "List of Priority Pathogens for Research and Development of New Antibiotics" [12]. According to the World Health Organization (WHO), antibiotic resistance is one of the biggest public health issues as around 79% of bacteria have developed resistance to at least one antibiotic. An estimated 700,000 people worldwide die each year from drug-resistant bacterial illnesses, and researchers estimate that figure will increase to 10 million by 2050 [13]. In the United States alone, antibiotic resistance costs healthcare systems around $20 billion a year.

Antibiotic resistance is mostly caused by genetic changes in bacteria or the introduction of genetic material from other organisms, such as viruses, other bacteria, or the environment [14]. Furthermore, between 40 and 80 percent of bacteria are capable of forming biofilms [15]. Communities of sessile microorganisms that have adhered to a surface or to one another to form a polymeric matrix make up biofilms. After being inserted into the matrix, bacterial cells alter their pace of growth and the transcription of their genes. Apart from demonstrating substantial gene exchange, which raises the rate of recombination across strains, they also contribute specific proteins that contribute to their resistance [16, 17]. According to the National Institute of Health, up to 80% of all microbial infections in humans are caused by endocarditis, cystic fibrosis, meningitis, kidney infections, chronic wounds that do not heal, and infections related to implanted devices such as heart valves, prosthetic joints, and urinary catheters [18].

The host immune system responds to a biofilm infection in both an innate and an acquired manner. Neither of these immune responses can destroy the pathogen in the biofilm because of the structural barrier provided by the polymeric matrix. Sessile bacteria are less responsive to standard antibiotic therapy because they are 500–5000 times more drug-resistant than planktonic cells. Therefore, new strategies are needed to stop biofilms from forming and to get rid of biofilms that already exist [19].

Other bacteria that are resistant to multidrug-resistant (MDR) antibiotics include methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant E. faecium (VRSA); polymyxin-resistant E. coli; and aminoglycoside-resistant Acinetobacter spp. [20]. The FDA approved nine additional antibiotics from 107 compounds in 2018 and 2019 to fight germs that are resistant to drugs [21].

Research on the genetic and phenotypic alterations caused by antibiotic resistance has been extensive [22]. Even though some promising research has been done on possible agents, it usually takes a long time to create new active antibiotic molecules [23]. Nevertheless, there is an urgent need for novel antibiotics. Therefore, reducing the problem of antibiotic resistance in a short period of time will be challenging.

The discovery of penicillin has led to a decrease in the usage of certain metals, oxides, or metallic salts for the treatment of bacterial and fungal illnesses. Recent years have seen a surge in the utilization of inorganic materials, and in particular, nanostructured systems, to fight dangerous microorganisms [24]. One proposed antibiotic is a metal or metal oxide nanoparticle, such as titanium dioxide (TiO2), magnesium oxide (MgO), zinc oxide (ZnO), silver (Ag), or gold (Au).

Discovery of gold and silver nanoparticles (Au NPs and Ag NPs, respectively) has been spurred by the possibility of a new class of antibiotics. The bacteria that these NPs have eliminated include E. coli [25], Staphylococcus aureus [26], Pseudomonas aeruginosa [27], Proteus vulgaris, S. aureus, Proteus mirabilis, Enterobacter cloacae, and Staphylococcus epidermidis. These metallic NPs have the ability to inhibit bacterial growth by either eradicating dangerous germs or preventing the formation of bacterial biofilms.

Both the external and internal components of bacteria can be harmed by Ag NPs due to their broad-spectrum. The reducing agent's characteristics and the kind of stabilizer have a significant impact on the surface characteristics of Ag NPs, which can be customized using a range of synthesis techniques [28, 29]. Nanoparticles' (NPs') effects on bacterial membranes depend on their physical and chemical properties, such as their size, shape, surface area, charge, oxidation state, and surface chemistry. Ag NPs that are small and colloidally stable are better than those that are prone to aggregation [30].

Size is a key consideration in the interactions of NPs with cells. The interaction between the Ag NPs is influenced by their size. Several studies have shown that Ag NPs with a diameter of 3-10 nm are the most effective at killing bacteria because of their favorable direct connection with the bacterial membrane [31] and the speed at which bacteria are killed following their engagement. Because Au NPs have antimicrobial qualities, neither bulk nor ionic gold have them. Gold nanoparticles (Au NPs), for instance, can stop bacteria from metabolizing by breaking down their membranes. NPs' small size may make gold colloid more susceptible to NP aggregation. It's typical practice to stabilize Au NPs by adding things like polymers or polyelectrolytes [32]. Such stabilizing compounds, which function as capping or protective agents, prevent aggregation due to steric hindrance [33].

The size and form of Au NPs, two essential characteristics, have been altered and studied in order to improve the hydrogel's antibacterial activity. This was demonstrated by research that produced nanospherical of 29.2 nm [34], nanorods of 82.5 nm, 54 nm [35], and 49.2 nm [36], and nanostars with a core diameter of 25 nm and average sizes of 50 nm, 70 nm, and 120 nm [37].

2. LITERATURE REVIEW

Aguilar-Garay et al. (2024) [38] evaluated research that was published in prestigious journals in the domains of materials science, biomedical research, and nanotechnology between 2006 and 2023. The main uses examined are the synthesis techniques, morphological traits, and modes of action of Ag and Au NPs, as well as their efficacy as antibacterial agents. A comprehensive review of the literature on the synthesis, morphology, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and efficacy of Au and Ag NPs against various Gram (+/−) bacteria has validated their antibacterial activities. The way that NPs are produced and their characteristics—such as size, shape, and surface charge—all affect how well they interact with bacterial cells and how effective they are against bacteria. They want to develop NP-based antimicrobial techniques by identifying the properties of Ag and Au NPs that enhance their antibacterial activity. This study highlights the low cytotoxicity of Ag and Au NPs as well as their inhibitory effects against various bacterial species. These materials are therefore positioned as promising candidates for the creation of antibacterial drugs, which will significantly help the global effort to combat illnesses that are resistant to antibiotics.

Ghasemi et al. [39] examined the response surface approach in their 2024 study to maximize the environmentally friendly production of AgNPs utilizing an extract from R. discolor leaves. In order to produce AgNPs, the optimal conditions were 7.11 mM AgNO3, 17.83 hours, 56.51 °C, and 29.22% extract as a percentage. The production of AgNPs was confirmed by UV-visible spectroscopy (λmax at 456.01 nm). AgNPs with an average size of 37 nm were found to be uniformly distributed in a TEM examination. XRD was used to confirm the structure. At 3 keV, the EDX showed a clear peak that was determined to be Ag. It was concluded that the nanoparticles were stable because the zeta potential value was negative, at 44.2 mV. FT-IR spectra showed the presence of plant molecules with functional groups, which are essential for bio-reduction and capping. The AgNPs showed microbiological inhibitory concentrations (MICs) between 0.93 and 3.75 mg ml−1 against Pseudomonas aeruginosa and multidrug-resistant Escherichia coli. The phytochemical study's findings demonstrated that the surface of AgNPs was composed of flavonoids, tannins, and phenolics. Furthermore, they demonstrated significant cytotoxic effects on HepG2, MCF-7, and A431 cells, with IC50 values ranging from 11.1 to 49.1 µg ml−l.

Balciunaitiene et al. (2024) [40] evaluated the production of AgNPs using scanning electron microscopy-energy-dispersive spectroscopy (SEM-EDS) and transmission electron microscopy (TEM). The researchers limited the growth of both Gram-positive and Gram-negative bacterial cultures using the Kirby-Bauer disk diffusion susceptibility test to assess the biofilm's antimicrobial activity. The green AgNPs were applied to the biofilm. Sym. Radix aqueous extracts exhibited slightly higher total phenolic content and antioxidant activity compared to Sym. Radix/AgNPs. Against certain test bacterial cultures, polymer film/AgNPs had a significantly greater antibacterial effect than pure film. AgNPs were found to include spherical nano-objects with an average size of 27.45 nm, according to transmission electron microscopy pictures. According to the SEM-EDS analysis results, the metal nanoparticles were uniformly distributed throughout the biopolymeric matrix. Morphological analysis of the films showed a smooth, flat surface free of holes and other relief flaws.

Sarma et al. (2024) focused especially on antibacterial gold nanoparticles [41]. Antimicrobial resistance (AMR) is a severe issue brought on by the overuse and abuse of antibiotics, and it presents a significant risk to the efficient treatment of the bacterial infections that are becoming more and more common. In addition to their poor penetration and low bioavailability, conventional antibiotics have other drawbacks, including the emergence of antibiotic-resistant microorganisms. Recent developments in nanotechnology, which enable the introduction of nanoparticles with intriguing physicochemical features, have raised expectations for a novel defense against diseases resistant to antibiotics. The numerous benefits of employing nanoparticles include enhanced tissue targeting, solubility, stability, and epithelial permeability, along with minimal side effects. Except for gold nanoparticles (AuNPs), the majority of metal nanoparticles still have significant problems with their biological safety. AuNPs' many biological activities, such as their antiviral, antifungal, anti-inflammatory, and antibacterial properties, as well as their low toxicity, biocompatibility, chemical stability, and functional flexibility, make them a desirable option for drug delivery and medical applications.

Kazem et al. (2024) [42] investigated the antibacterial properties of bio-synthesized gold nanoparticles in connection with methicillin-resistant bacterial strains. Antibiotic-resistant genes are hard, if not impossible, to remove from a bacterial colony once they have established themselves. Additionally, resistance is steadily rising. These genes become an essential part of the bacterial genome after integration. As novel mechanisms of resistance are identified, it is getting more and more difficult to provide a treatment that may successfully address this condition that is resistant to several medications. Potential causes of antibiotic resistance include the manufacturing of medications, wastewater treatment, and the use of antibacterial substances in personal hygiene products. Potential exposures include ingesting contaminated water, coming into contact with ill farmhands or meat processors, and air pollution from animal housing and transportation. These circumstances demonstrate the urgent need for innovative and novel methods to the treatment of infectious diseases as well as the need for improvements to the technology and procedures used in

drug development today. The goal of the research priority should be to find a long-term solution to these resistance problems, which can and will act as a basis for further medical research in these areas. Ibraheem et al. (2024) examined the efficacy of Nystatin (NYS) as an antibacterial drug against two bacterial species: Staphylococcus aureus and Escherichia coli [43]. Silver nanoparticles (AgNPs) were produced by a chemical reduction process and subsequently loaded with the NYS. AgNPs and NYS were combined with polyethylene glycol-300 (PEG) to generate the unique nanocomposite, known as AgNPs-PEG-NYS. The generated AgNPs, AgNPs-NYS, and AgNPs-PEG-NYS were analyzed using Fourier-transform infrared spectroscopy, X-ray diffraction, UV-visible spectrophotometry, and transmission electron microscopy. Using UV-visible spectrophotometry, three distinct wavelengths—426 nm, 421 nm, and 408 nm—were discovered. X-ray diffraction demonstrated high crystallinity, and Fourier-transform infrared spectroscopy was used to ascertain their characteristics. Transmission electron microscopy demonstrated the spherical forms of AgNPs and their conjugates. The average size of AgNPs, AgNPs-NYS, and AgNPs-PEG-NYS, respectively, is 37.658 nm, 52.328 nm, and 71.525 nm, according to the size distribution. The well-diffusion approach was employed to ascertain if AgNPs and their conjugates hindered the growth of infectious bacteria. The results of our investigation verified that AgNPs-NYS exhibited improved antibacterial capabilities. The inhibition zone diameters for S. aureus and E. coli were 19.52 mm and 17.54 mm, respectively, indicating that the AgNPs-PEG-NSY nanocomposite exhibited superior inhibitory action in comparison to other nanoparticles that were synthesized.

González-Ballesteros et al. (2023) [44] employed an aqueous extract of the invasive macroalgae Undaria pinnatifida (UP) to perform the first green synthesis of gold and silver nanoparticles. I defined the nanoparticles using a variety of physicochemical techniques. The average size of the crystalline Ag@UP and spherical Au@UP was 14.1 ± 2.8 nm and 6.8 ± 1.0 nm, respectively. Proteins and carbs from the UP extract may be used in the manufacturing and capping of the nanoparticles. The UP extract, Ag@UP, and Au@UP were tested for their antimicrobial activity against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans, and Candida auris. Ag@UP exhibited the greatest antibacterial activity with exceptionally low MIC and MBC values for every tested bacterium. Conversely, Au@UP shown exceptional efficacy against bacteria that form biofilms. Ag@UP and Au@UP shown exceptional antifungal activity by inhibiting the development of hyphae. This study indicates that this invasive brown alga has significant potential for use in biomedical applications.

More et al. (2023) [45] explored several ways to produce silver nanoparticles (AgNPs), such as how they change from an elemental state to a particle format and how they fight bacteria that can build biofilms and are resistant to many medications. AgNPs kill bacteria by causing oxidative stress, protein dysfunction, membrane rupture, and DNA damage, according to numerous studies. Furthermore, by altering the adhesion of bacterial cells, AgNPs can prevent the formation of biofilms. The medical advantages of using AgNPs are slightly outweighed by their detrimental effects on humans and the environment. Here, we review the latest research on the antibacterial qualities of AgNPs and discuss the different ways in which these nanoparticles fight off dangerous microorganisms. We provide a brief overview of the ongoing AgNP clinical trials. It is particularly important to comprehend how AgNPs affect bacterial biofilms, which are a major contributor to pathogenicity. AgNPs are used in medicine, but they also have certain non-medical applications, like wound healing promotion and diagnostics.

Alotaibi et al. (2022) [46] assessed combining traditional antimicrobials with AgNPs would produce a synergistic response, established the optimal and safe minimum inhibitory concentration (MIC) range against a variety of wild-type Gram-positive and -negative strains as well as three different clinical isolates of AMR Klebsiella pneumoniae. The cytotoxicity of the synergistic combinations was assessed using a different human hepatocyte model. The results showed that AgNPs (15–25 nm) had no effect on Gram-positive strains, with a MIC value of 256 µg/mL, but they had an impact on Gram-negative bacteria, with MIC values ranging from 16–128 µg/mL. Following exposure to AgNPs, the MIC values of AMR and wild-type Klebsiella pneumoniae were similar. Antimicrobial medications such as kanamycin, colistin, rifampicin, and vancomycin, when administered combined, shown

synergy against both wild-type and AMR K. pneumoniae isolates; however, vancomycin did not function against the AMR strain. It should be noted that the combinations under investigation exhibited negligible to no toxicity to hepatocytes.

Ali et al. (2020) demonstrated that conjugation with cefixime enhanced the cytocompatibility of gold and silver nanoparticles [47]. CEfixime-conjugated gold and silver nanoparticles (Cfm-AuNPs and Cfm-AgNPs, respectively) were tested against S. aureus (S. aureus) ATCC 25923. The generated nano-conjugates were examined using infrared (IR), ultraviolet-visible (UV-VIS), and atomic force microscopy (AFM) techniques. We compared the bactericidal activity of Cfm-AuNPs and Cfm-AgNPs to that of non-conjugated cefixime as a control. Experimental results showed that conjugation with gold and silver NPs boosted cefixime's bactericidal potential by 8 and 3 times, respectively. Furthermore, the conjugated cefixime demonstrated improved antibacterial activity and kinetics under AFM. Surface topography measurements of both treated and control S. aureus cells show that conjugation with either gold or silver NPs reduces the effective treatment time of cefixime from eight hours to four hours.

3. METHODOLOGY

3.1 Selection and Preparation of Nanoparticles

To assess the antimicrobial ability of AuNPs and AgNPs, it is essential that nanoparticles be obtained or synthesized reproducibly and consistently in terms of particle size, shape, surface charge, and concentration. Typically, most nanoparticles are synthesized by chemical reduction techniques involving the reduction of metal salts; these may include gold chloride or silver nitrate into stable nanoparticles. Concentrations of reducing agents and stabilizers are controlled in such a way that it results in a narrow distribution of the size of synthesized nanoparticles because size and shape affect antimicrobial activity. After the nanoparticles are prepared, the characterizations of the nanoparticles would be conducted using methods such as UV-Visible spectroscopy, dynamic light scattering (DLS), and TEM to ascertain the morphological confirmation and the sizing and stability of the particles. These nanoparticles are then dispersed in a biocompatible medium such as, for instance, deionized water or phosphate-buffered saline in order to enable them to be used in biological tests.

3.2 Pathogen Selection and Culturing

A range of MDR bacterial isolates is tested for the antimicrobial efficacy of AuNPs and AgNPs. The experiment took some of the most clinically relevant common MDR pathogens, Methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, and Escherichia coli, due to their clinical relevance and well-studied resistance patterns against many antibiotics. All pathogens are always cultured under sterile conditions in nutrient-rich media, preferably tryptic soy broth or agar, so as not to lose their virulence and resistance. Standardization of bacterial cultures involved the adjustment of cell densities to about 10^6 CFU/mL using optical density measurements at 600 nm or serial dilution; this is the critical step to the reproducibility of the antimicrobial testing.

3.3 Experimental Design for Antimicrobial Testing

• **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Testing**: MIC and MBC are tests that determine the minimum concentration of AuNPs and AgNPs inhibiting and killing bacterial pathogens. In MIC tests, a 96-well plate is filled with serial dilutions of nanoparticles with standard concentrations of bacterial cells in each well. Plated is incubated for 24 hours at 37°C. At the end of this incubation, bacterial growth is visualized by the naked eye, or optical density is measured. The MIC is recorded as the lowest concentration at which there is no visible growth. For MBC determination, the samples from wells at and above the MIC are plated on agar and then incubated to determine the lowest nanoparticle concentration at which no bacterial colony forms, which is an indicator of bactericidal effect.

• **Agar Diffusion Assays**: In this technique, a lawn of bacterial culture is spread on an agar plate and wells or discs loaded with different concentrations of AuNPs and AgNPs. Plates are incubated for 18-24 hours, following which the zones of inhibition-the clear areas around the wells or discs-are

measured. The bigger the zone of inhibition, the more significant the antimicrobial activity, and nanoparticle efficacy can be qualitatively compared.

3.4 Mechanism of Action Analysis

Understanding the mechanism of action of antimicrobial activities by AuNPs and AgNPs is important in their application as potential drugs. Advanced imaging techniques such as TEM and SEM are applied to image interaction between nanoparticles and bacterial cells. TEM and SEM images show adhesion of nanoparticles to the bacterial cell walls, internalization of the nanoparticles, and changes in morphology, such as disruption of the membranes. Biochemical assays are also carried out to estimate oxidative stress since AgNPs specifically have been known to induce the generation of ROS by bacterial cells. High concentrations of ROS can lead to destruction of membranes, oxidation of proteins, as well as DNA damage and results in cell death of the bacterial cell. Elaborative microscopy and nucleic acid-binding dyes further may reflect whether nanomaterials interact directly with the DNA of the bacteria to induce genotoxic effects.

3.5 Evaluation of Antibacterial Efficacy

After the incubation, activity of both AuNPs and AgNPs is assessed by counting CFU on the agar plates or by optical density in broth cultures. CFU directly measures viable bacteria, whereas optical density is a quick indirect measure for monitoring bacteria growth in liquid media. The data collected from both MIC and MBC and agar diffusion assays is analyzed to compare these nanoparticles' bactericidal and inhibitory effects on each of the pathogens. This information is crucial in establishing dose-response relationships and determining whether a particular type of nanoparticle, such as AgNPs, is significantly more potent as an antimicrobial than any other, like AuNPs.

3.6 Comparative Analysis with Standard Antibiotics

To contextualize the antimicrobial activity of nanoparticles, control tests are performed using the conventional antibiotics, such as penicillin, vancomycin or tetracycline, on the same bacterial strains. These will be benchmark tests and evaluate the relative efficacy of AuNPs and AgNPs. Of very significant interest to the performance of nanoparticles against MDR pathogens is the evidence of their potential to combat infections where traditional antibiotics fail to get the job done. Thus, the information drawn from such comparative analyses helps one identify whether nanoparticles might be a supplementary or alternative treatment in drug-resistant bacterial infections.

3.7 Data Analysis and Statistical Evaluation

The effectiveness of AuNPs and AgNPs against the MDR pathogens then comes to be determined through statistical methods. Analytical methods like ANOVA or Student's t-tests are used by comparing the zones of inhibition, MIC values, and MBC values for treatments and control groups. Significance levels, such as $p<0.05$, are used to deem whether the differences found have been of reliable origin, and the results are, therefore, represented as mean values with standard deviations. Graphical representations, including bar graphs or dose-response curves, have been used to graphically compare nanoparticle effectiveness among the different tested bacterial strains and types of nanoparticles.

3.8 Safety and Toxicity Assessment

To evaluate the nontoxic concentration of nanoparticles, which is effective against bacteria but not harmful to human cells, the acute toxicity of AuNPs and AgNPs is assessed for any clinical applications. Cell viability assays are performed in human tissue-derived cell lines like keratinocytes or fibroblasts to determine the viability of cells post exposure to nanoparticles. Quantitative estimations of cell health and cytotoxicity can be made through various types of assays such as MTT or LDH assay. This assessment ensures that the nanoparticles are safe for consideration as a future therapeutic by identifying concentrations that are both antimicrobial and biocompatible.

4. RESULT AND DISCUSSION

Table 1 and Fig. 1 exhibit the MIC and MBC value of gold (AuNP) and silver (AgNP) nanoparticles for the inhibition against three pathogens, namely MRSA, E. coli, and Pseudomonas aeruginosa. The MIC and MBC for the AuNPs were 16 µg/mL and 32 µg/mL, while in case of AgNPs, the difference is more pronounced with MIC value of 4 µg/mL, and the MBC value of 8 µg/mL. Similarly, for E. coli also, gold nanoparticles require higher concentrations to give almost similar inhibitory and bactericidal effects as compared to that of silver nanoparticles (MIC 20 µg/mL, MBC 40 µg/mL) compared to the latter. Pseudomonas aeruginosa follows the same trend with the result that the only MIC and MBC values are 25 µg/mL and 50 µg/mL, respectively, for the gold nanoparticles, whereas silver nanoparticles manifested lower values (MIC 8 µg/mL, MBC 16 µg/mL) to show a higher activity. In summary, the silver nanoparticles displayed a higher enhanced antimicrobial activity against all the used pathogens with more constant low MIC and MBC values as compared to the gold nanoparticles.

Table 1: Minimum Inhibitory and Minimum Bactericidal Concentration

Pathogen	Nanoparticle Type	MIC (µg/mL)	MBC (µg/mL)
MRSA	Gold (AuNP)	16	32
	Silver (AgNP)	4	8
E. coli	Gold (AuNP)	20	40
	Silver (AgNP)	5	10
Pseudomonas aeruginosa	Gold (AuNP)	25	50
	Silver (AgNP)	8	16

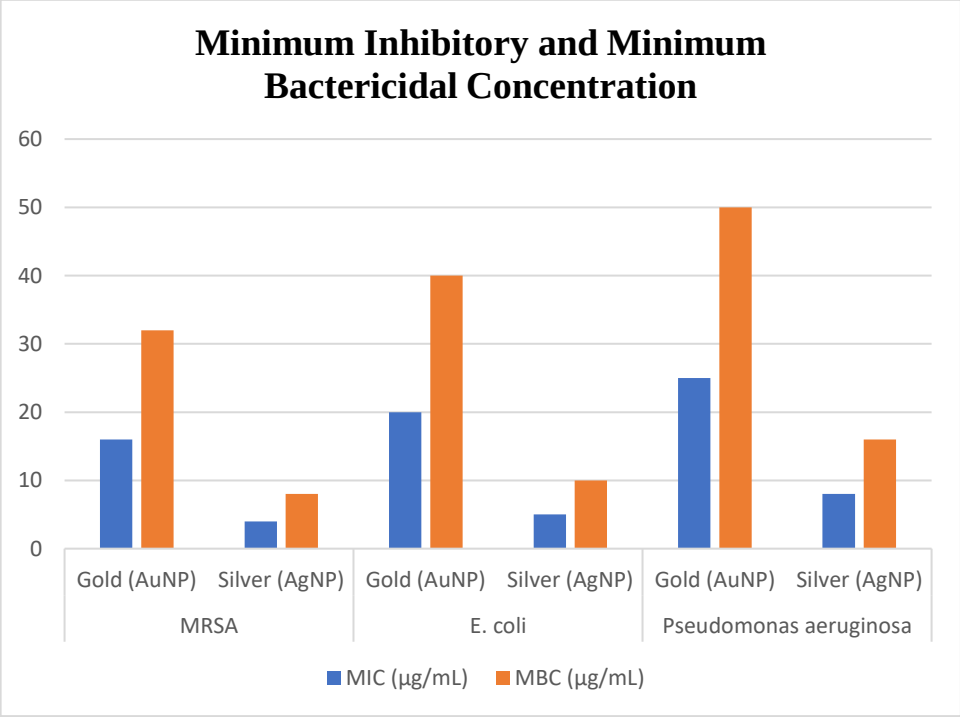

Fig. 1: Minimum Inhibitory and Minimum Bactericidal Concentration

Table 2 and Fig. 2 demonstrate the measured zone of inhibition in millimeters for the gold (AuNP) and silver (AgNP) nanoparticles against three pathogens: MRSA, E. coli, and Pseudomonas aeruginosa, with concentrations at 10 µg/mL and 20 µg/mL. For MRSA, AuNPs produce inhibition zones of 12 mm at 10 µg/mL and 16 mm at 20 µg/mL concentrations, whereas AgNPs produced significantly larger inhibition zones of 18 mm and 22 mm, respectively, for the same concentrations, suggesting higher antibacterial activity. For E. The gold nanoparticles inhibit the growth of coli. Inhibition zones of 10 mm and 14 mm are obtained while silver nanoparticles achieve 16 mm and 20

mm at 10 µg/mL and 20 µg/mL, respectively, thus showing the better efficacy of silver again. In case of Pseudomonas aeruginosa, the trend is continued by gold nanoparticles creating zones of 8 mm and 12 mm while, silver nanoparticles were producing zones of 14 mm and 18 mm. All the pathogens and concentrations show large zones of inhibition by silver nanoparticles compared to that of gold nanoparticles, which indicates that silver nanoparticles have a higher potency in antimicrobial activity.

Table 2: Agar Diffusion Assay - Zone of Inhibition

Pathogen	Nanoparticle Type	Concentration (µg/mL)	Zone of Inhibition (mm)
MRSA	Gold (AuNP)	10	12
		20	16
	Silver (AgNP)	10	18
		20	22
E. coli	Gold (AuNP)	10	10
		20	14
	Silver (AgNP)	10	16
		20	20
Pseudomonas aeruginosa	Gold (AuNP)	10	8
		20	12
	Silver (AgNP)	10	14
		20	18

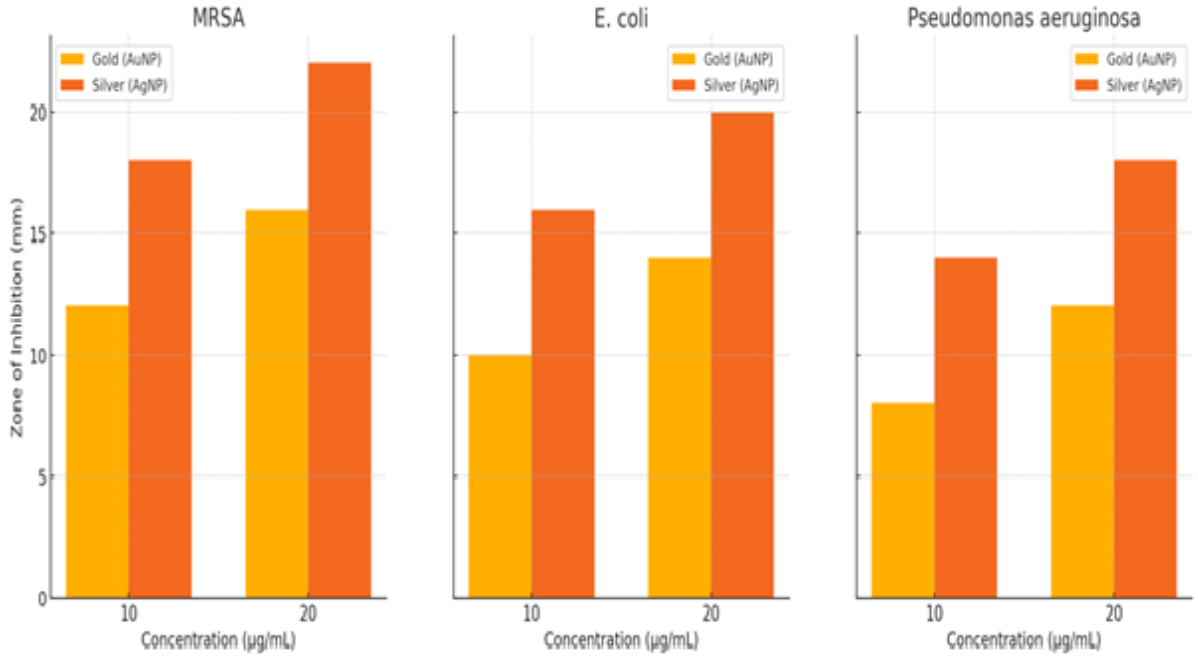

Fig. 2: Agar Diffusion Assay - Zone of Inhibition

Table 3 and Fig. 3 show the ROS production in RFU for bacterial cells treated by nanoparticles of gold (AuNP) and silver (AgNP) for three pathogens: MRSA, E. coli, and Pseudomonas aeruginosa. The gold nanoparticles had a generation of 500 RFU for MRSA, whereas for silver nanoparticles the ROS was significantly higher at 1200 RFU, which makes it have a more intense oxidative stress effect. In E. coli, ROS generation by gold nanoparticles is 450 RFU, while silver nanoparticles again prove with a higher value of 1100 RFU. Likewise, for Pseudomonas aeruginosa, it can be observed that ROS generation with gold nanoparticles is 400 RFU compared to 1000 RFU with silver nanoparticles. Across all pathogens, silver nanoparticles consistently induced greater ROS levels than gold

nanoparticles, suggesting that silver nanoparticles are capable of provoking more severe oxidative stress in the cells of the bacteria, possibly one reason why they presented more potent antimicrobial activity.

Table 3: Reactive Oxygen Species (ROS) Generation in Bacterial Cells

Pathogen	Nanoparticle Type	ROS Generation (Relative Fluorescence Units - RFU)
MRSA	Gold (AuNP)	500
	Silver (AgNP)	1200
E. coli	Gold (AuNP)	450
	Silver (AgNP)	1100
Pseudomonas aeruginosa	Gold (AuNP)	400
	Silver (AgNP)	1000

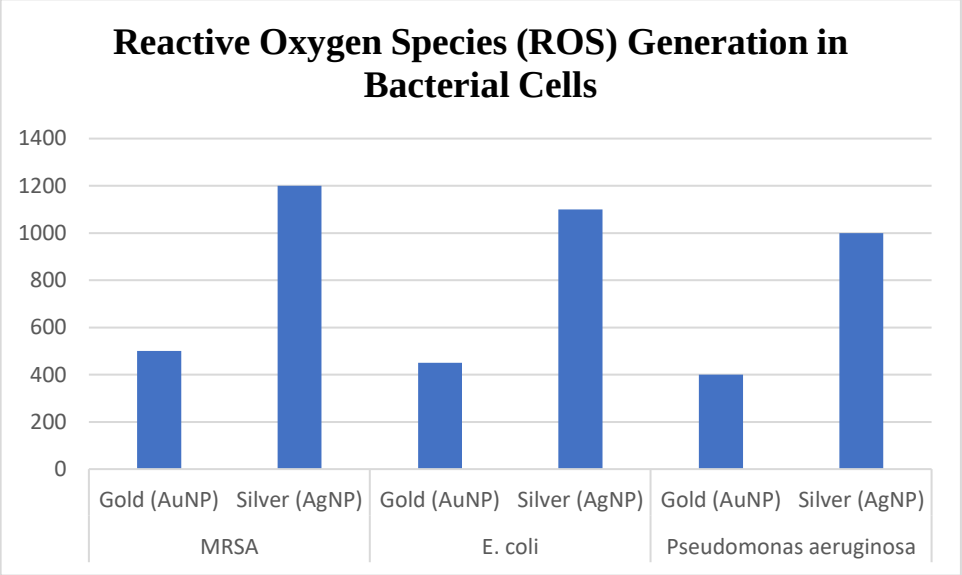

Fig. 3: Reactive Oxygen Species (ROS) Generation in Bacterial Cells

Table 4 and Fig. 4 display a comparison of antimicrobial activity of AuNP and AgNP against MRSA, E. coli, and Pseudomonas aeruginosa with standard antibiotics, in terms of MIC in µg/mL and zone of inhibition in millimeters. The MIC of gold nanoparticles for MRSA was 16 µg/mL and the inhibition zone was at 16 mm while that of silver nanoparticles had the best effectiveness with a MIC of 4 µg/mL and a zone of 22 mm. Meanwhile, the comparison of the difference is evident on the part of penicillin, with a MIC of 64 µg/mL and an inhibition zone of 10 mm, and vancomycin that has the least MIC of 2 µg/mL and the largest inhibition zone of 24 mm. For E. coli, the AuNPs showed a MIC of 20 µg/mL and an inhibition zone of 14 mm, whereas the AgNPs were more potent with a MIC of 5 µg/mL and an inhibition zone of 20 mm. Tetracycline was also comparable and produced a MIC of 8 µg/mL and an inhibition zone of 18 mm. Among them, gold nanoparticles exhibited MIC of 25 µg/mL and gave a 12 mm zone against Pseudomonas aeruginosa whereas silver nanoparticles displayed better action with an MIC of 8 µg/mL and an 18 mm zone. Conversely, the action of ciprofloxacin as an antibacterial was highest at the MIC level of 1 µg/mL with the largest inhibition zone of 28 mm.

Table 4: Comparative Efficacy with Standard Antibiotics

Pathogen	Treatment Type	MIC (µg/mL)	Zone of Inhibition (mm)
MRSA	Gold (AuNP)	16	16
	Silver (AgNP)	4	22
	Penicillin	64	10
	Vancomycin	2	24
E. coli	Gold (AuNP)	20	14
	Silver (AgNP)	5	20
	Tetracycline	8	18
Pseudomonas aeruginosa	Gold (AuNP)	25	12
	Silver (AgNP)	8	18
	Ciprofloxacin	1	28

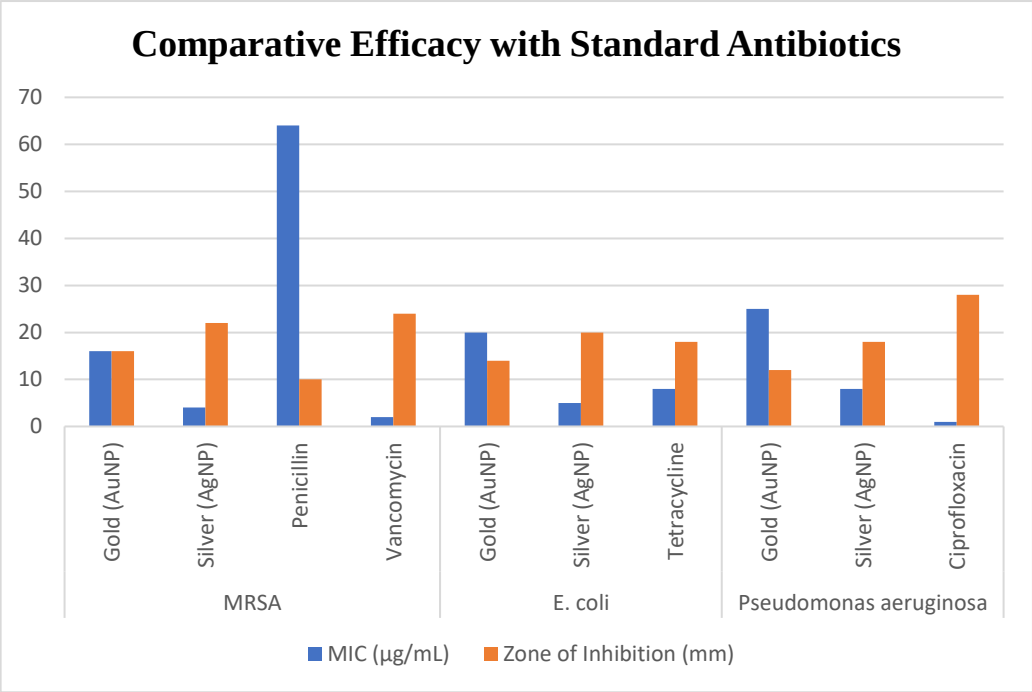

Fig. 4: Comparative Efficacy with Standard Antibiotics

Table 5 and Fig. 5 show the cell viability of the cytotoxicity of gold (AuNP) and silver (AgNP) nanoparticles in human cell lines (Keratinocytes and Fibroblasts) at different concentrations, i.e., 10 µg/mL and 20 µg/mL. The cell viability in the case of keratinocytes is found to be 95% at 10 µg/mL and at 20 µg/mL concentration, and it is 90% in the case of gold nanoparticles; hence, it shows negligible toxicity. However, silver nanoparticles depicted low viabilities of 85% at 10 µg/mL and 70% at 20 µg/mL, which indicates higher cytotoxicity. In the case of fibroblasts, gold nanoparticles showed cell viability at 96% and 92% for 10 µg/mL and 20 µg/mL, respectively, indicating good biocompatibility. Again, silver nanoparticles exhibit high toxicity as there was cell viability at 80% for 10 µg/mL and 68% for 20 µg/mL.

Table 5: Cytotoxicity Assessment on Human Cell Lines

Cell Line	Nanoparticle Type	Concentration (µg/mL)	Cell Viability (%)
Keratinocytes	Gold (AuNP)	10	95
		20	90
	Silver (AgNP)	10	85
		20	70
Fibroblasts	Gold (AuNP)	10	96

		20	92
	Silver (AgNP)	10	80
		20	68

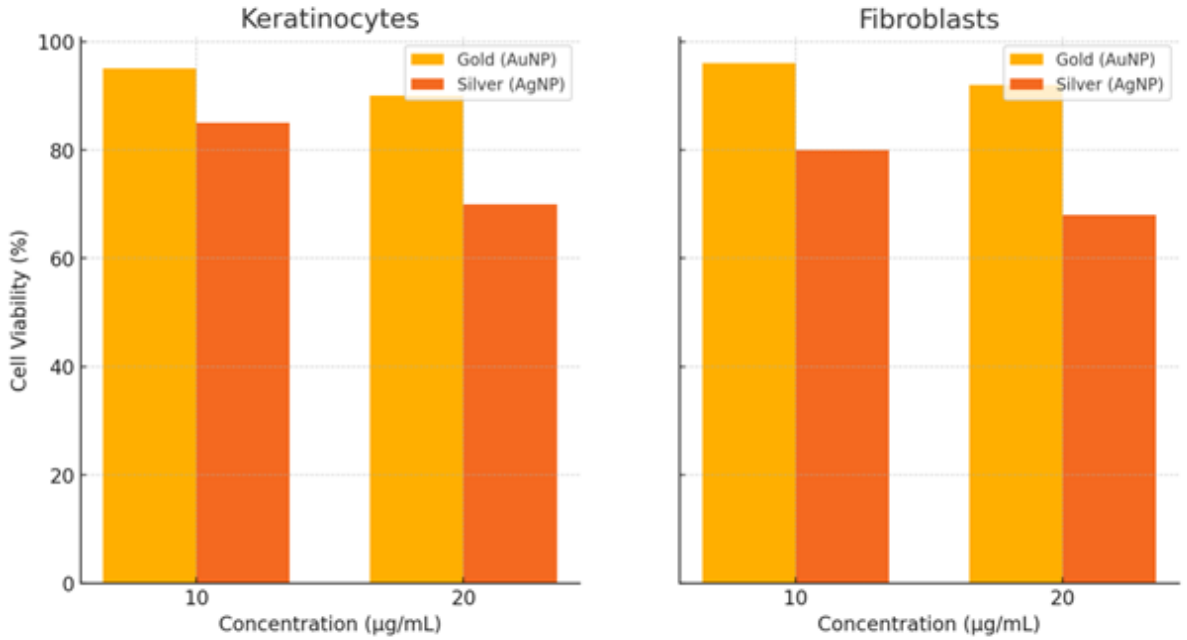

Fig. 5: Cytotoxicity Assessment on Human Cell Lines

5. CONCLUSION

In conclusion, both AuNP and AgNP exhibited excellent antimicrobial activities against multi-drug-resistant strains while the latter appeared to be the most intense. The results obtained in this work demonstrate that AgNPs have relatively lower MIC and MBC values along with an extended zone of inhibition than those of AuNPs, thus implying better antimicrobial activity. The higher level of generated ROS levels by AgNPs suggests that the mechanism that is involved in enhanced bactericidal activity is oxidative stress. Even though these results highlight that AgNPs hold immense promise as effective alternatives to traditional antibiotics, assessment in terms of cytotoxicity questions the need for optimal dosage which can help reduce adverse effects on human cells. Thus, the silver nanoparticles present a very promising avenue for developing new treatments against drug-resistant infections, if their safety profile is well managed for clinical use.

Declaration of competing interest

There is no conflict of interest.

Funding declaration

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Ethics declaration

Not applicable.

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