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RESEARCH

Comparison of Multi-metallic Nanoparticles-Alternative Antibacterial Agent: Understanding the Role of Their Antibacterial Properties

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Abstract

The rise of antibiotic-resistant infectious diseases caused by various bacterial pathogens has emerged as a major global health concern. As a result, there has been a growing effort to develop innovative antimicrobial materials as an alternative solution to combat multidrug-resistant (MDR) bacteria. Among these materials, metal nanoparticles (MNPs), particularly multi-metallic nanoparticles (MMNPs), have been found to demonstrate promising potential in fighting against antimicrobial resistance. The unique physiochemical properties and excellent biocompatibility of MMNPs contribute to their remarkable antimicrobial activity. MMNPs, composed of multiple metals, exhibit diverse electronic, optical, and magnetic properties. These multifunctional characteristics, including size, shape, surface area to volume ratio, and surface charge potential, facilitate favorable interactions with bacterial cell membranes. Consequently, MMNPs can disrupt the bacteria cell membrane, metal ion release, biomolecule damage, induce the generation of reactive oxygen species (ROS), cause protein dysfunction, and inflict DNA damage within the bacterial host's environment. This scientific review aims to provide a comprehensive summary and comparison of research progress concerning the antibacterial activities of multi-metallic nanoparticles, as well as their synergistic effects. Additionally, the scientific review elucidates the mechanisms through which MMNPs exert their antibacterial effects. Significant emphasis has been placed on recent promising advances of MMNPs that aid in overcoming antibacterial resistance. The physiochemical and multifunctional properties of MMNPs play a pivotal role in determining their effectiveness against bacterial infections. By integrating current knowledge on the antibacterial activities of MMNPs, this scientific review offers valuable insights into the potential applications of MMNPs in combating bacterial infections.

Keywords Infectious diseases · Antibiotics · Antibacterial materials · Synergistic effect · Multi-drug resistance · Multi-metallic nanoparticles · Antibacterial activity

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1 Introduction

Pathogenic bacteria continue to pose a significant threat to human health worldwide, and the emergence of antimicrobial-resistant (AMR) strains has further exacerbated adverse effects, causing/even death in extreme situations. Antibiotics, which have long been the cornerstone of bacterial infection treatment, are increasingly becoming ineffective due to the development of multidrug-resistant (MDR) bacteria. The overuse and misuse of antibiotics have led to the selection and proliferation of resistant strains, rendering conventional treatments ineffective against these pathogens. Consequently, the World Health Organization (WHO) has recognized that there is an urgent need for an improved and coordinated effort to combat the antimicrobial resistance [1, 2]. It is urgently required to explore alternative appropriate new drug materials to combat antibiotic-resistant bacteria and address the growing global health crisis caused by infectious diseases. The lack of adequate monitoring in many parts of the globe leaves a large gap in existing awareness of the distribution and extent of this antimicrobial resistance.

The infectious communicative diseases caused by the MDR bacteria strains are increasing at a dangerously alarming rate; among multi-antibiotic resistant bacterias, *methicillin*-resistant *Staphylococcus aureus*, *vancomycin*-resistant *Enterococcus faecium*, and *fluoroquinolone*-resistant *Pseudomonas aeruginosa* are of serious concern [3]. In addition, different foodborne pathogens associated with gram-positives (*Bacillus cereus*, *E. coli*, *S. aureus*) are causing a large number of infectious diseases with major influence on the public health and safety sector [4].

The production of rich antibacterial resistant antibiotics requires longer processing time, and it also causes toxic side effects. The global demand forces the research community to develop alternative antimicrobial resistant materials to treat infectious diseases without using the existing antibiotics. Metal Nanoparticles composed of more than two metals have several advantages, such as increased antibacterial activity due to synergistic effects, and reduced adverse effects of hybrid composite systems. In this approach, one antibacterial agent influences the other; they finally work more efficiently due to the different mechanisms of their individual constituent atoms. This might be a new methodology to solve global demand for bacterial resistant drugs and minimize the antibacterial sustainability. Recently, several studies confirmed the effective antibacterial activity observed with MMNPs using plant extracts [5].

Nanoparticles are tiny particles with dimensions between 1 and 100 nm (nm). They have unique physical and chemical properties due to their large surface area, high reactivity, and quantum effects. Nanoparticles can be made of different materials, such as metals, metal oxides, polymers, carbon, etc. Nanoparticles can also be composed of more than one material, forming bimetallic, tri-metallic or multi-metallic nanoparticles. These metal nanoparticles have different structures, such as alloys, core-shells, or contact aggregates. Metal nanoparticles have many potential applications in various fields, such as catalysis, energy, electronics, optics, sensors, etc. One of the most promising applications of metal nanoparticles is in the field of biotechnology, especially for antibacterial or antimicrobial purposes. Antibacterial or antimicrobial nanoparticles can kill or inhibit the growth of bacteria or microorganisms that cause infections or diseases. They can also enhance the efficacy of existing antibiotics or overcome the resistance mechanisms of bacteria.

In recent years, metal nanoparticles (MNPs) have been emerging as a promising candidate for the development of novel antimicrobial agents and solution against the resistance to the traditional multi-drug antibiotics [6]. MNPs -monometallic, bimetallic, trimetallic and multimetallic nanoparticles—possess unique physiochemical properties that make them attractive for antibacterial applications. The small size, large surface area to volume ratio, and distinct surface chemistry of MNPs contribute to their enhanced interactions with bacterial cells, enabling effective antimicrobial activity [7–9]. Additionally, the inherent antimicrobial properties of certain metals, such as silver, copper, gold, zinc, and titanium make them excellent candidates for antibacterial applications [10]. MNPs offer a potential solution to combat antibiotic resistance and address the limitations of conventional antibiotics by providing alternative mechanisms of action against bacteria [11–13].

Monometallic nanoparticles (MNPs), such as silver, copper, gold, zinc, and titanium nanoparticles demonstrated notable antibacterial properties. Silver nanoparticles (AgNPs) exhibit broad-spectrum antibacterial activity by disrupting bacterial cell membranes, inhibiting enzymatic processes, and inducing oxidative stress. They have shown efficacy against both Gram-positive and Gram-negative bacteria, including multidrug-resistant strains [14–19]. Copper nanoparticles (CuNPs) possess potent antibacterial effects due to their ability to release copper ions, which disrupt cell membranes, generate reactive oxygen species (ROS), and cause DNA damage [20, 21]. CuNPs have shown effectiveness against various bacterial pathogens, including antibiotic-resistant strains. Gold nanoparticles (AuNPs) exhibit antibacterial activity through multiple mechanisms, including membrane disruption, ROS generation, and protein inactivation. AuNPs have demonstrated efficacy against both Gram-positive and Gram-negative bacteria, although their activity against certain strains may be limited [22, 23]. Zinc nanoparticles (ZnNPs) exert antibacterial effects by

releasing zinc ions, which disrupt cellular processes and induce oxidative stress. ZnNPs have shown antibacterial activity against a range of pathogens, including antibiotic-resistant strains, making them promising candidates for antimicrobial applications [24–28].Titanium nanoparticles (TiNPs) have been studied for their antibacterial properties, mainly in the context of orthopedic and dental applications [29–32]. TiNPs can inhibit bacterial growth by interfering with their cellular processes and inducing oxidative stress, particularly against oral bacteria.

Multi-metallic nanoparticles (MMNPs) are new materials that incorporate more than two distinct metals to make hybrid materials with tunable multifunctional properties. MMNPs can be modified by controlling their size, shape, and morphology to achieve higher synergistic performance. Among monometallic nanoparticles, AgNPs are well-known for their ability to kill both Gram positive and Gram-negative bacteria and they even effectively work against multi-drug resistant species. Bi-, tri-, and MMNPs can be produced by the combination of two or more different types of metals to form a single new nano hybrid material with different structure, morphology and tunable material properties. Trimetallic nanoparticles involve the combination of three different metals, offering the opportunities for synergistic effects, resulting in enhanced antibacterial activity. Although studies on trimetallic nanoparticles are relatively limited compared to monometallic and bimetallic counterparts, there are some notable findings. Silver-copper-zinc nanoparticles (Ag–Cu–Zn NPs) have been found to possess enhanced antibacterial activity due to the collective effects of the three metals. Ag–Cu–Zn NPs can disrupt bacterial cell membranes, induce ROS generation, and cause DNA damage, resulting in increased bactericidal efficacy against a wide range of pathogens. Silver-gold-copper nanoparticles (Ag–Au–CuNPs) combine the unique properties of all three metals, resulting in synergistic antibacterial effects. Ag–Au–Cu NPs have shown improved antibacterial activity compared to monometallic or bimetallic counterparts, making them more potential candidates for advanced antimicrobial applications. These materials exhibit unique antibacterial properties. However, so far only monometallic, bimetallic, and very few trimetallic nanomaterials have been studied for their potential antibacterial effects.

In summary, while monometallic nanoparticles exhibit intrinsic antibacterial activities, bimetallic and trimetallic nanoparticles offer enhanced synergistic effects due to the collaborative effects of multiple metals, which provides opportunities for improved antibacterial efficacy, making them more promising candidates for the development of advanced antimicrobial agents against drug-resistant bacterial pathogens. The escalating issue of antibiotic resistance necessitates exploration of alternative antimicrobial agents. Metal nanoparticles offer a promising avenue due to their unique physicochemical properties and inherent antimicrobial capabilities. This review article aims to provide a comparative analysis of the antibacterial activities of monometallic, bimetallic, tri-metallic, and multi-metallic nanoparticles, highlighting their potential as effective antimicrobial agents. Elucidating the underlying mechanisms and exploring the diverse range of metal nanoparticles, this literature review aims to contribute the understanding of their antibacterial efficacy and pave the way for the development of targeted antibacterial strategies.

This literature review study also aims to compare the efficacy of these nanoparticles in terms of their antibacterial properties, highlighting the potential advantages of using multi-metallic nanoparticles over monometallic or bimetallic counterparts. This literature review will explore the underlying mechanisms through which MNPs exert their antibacterial effects, focusing on their physicochemical properties and their interactions with bacterial cells. This review summarizes the findings from the most recent literature on mono-,bi-, tri-, and multi metallic nanoparticles and provides insights into the comparative antibacterial abilities of different types of metal NPs.

In this article, we compared the antibacterial or antimicrobial activities or effects of monometallic, bimetallic, trimetallic and multi-metallic nanoparticles. We also discussed the factors that influence their antibacterial performance, such as size, shape, composition, structure, surface modification, concentration, pH, etc. The biogenic synthesis mono/bi/tri-metallic metal nanoparticles are shown in Fig. 1.

2 Physicochemical Properties of Metal Nanoparticles

2.1 Size and Shape

The size and shape of MNPs play a crucial role in determining their antibacterial activities. Smaller size nanoparticles have a larger surface area to volume ratio, resulting in increased interactions with bacterial cells. Different shapes of metal nanoparticles, such as nano-spheres, nano-rods, or nanowires, can influence the attachment and penetration of nanoparticles into bacterial membranes.

2.2 Composition

The composition of MNPs, whether monometallic, bimetallic, trimetallic, or multimetallic, significantly affects their antibacterial properties. The combination of different metals creates synergistic effects, altering their physiochemical properties and antibacterial mechanisms.

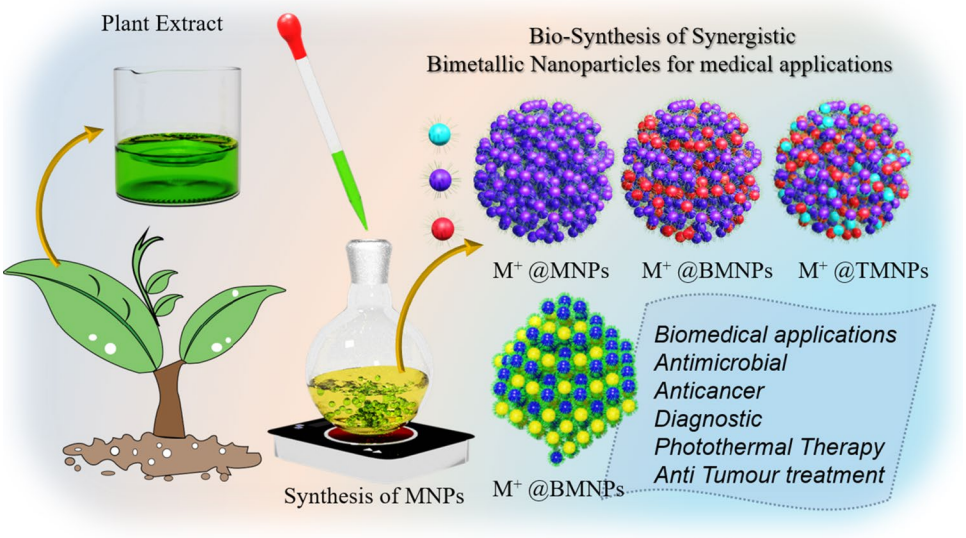

Fig. 1 Biogenic synthesis of plant leaf extract mediated MNPs

2.3 Surface Charge and Zeta Potential

The surface charge and zeta potential of MNPs influence their interactions with bacterial cells. The surface charge can determine the electrostatic interactions between nanoparticles and bacterial membranes, affecting their attachment, penetration, and subsequent antibacterial effects.

2.4 Surface Area to Volume Ratio

The high surface area to volume ratio of MNPs contributes to enhancing their antibacterial activities. A larger surface area allows for more interactions with bacterial cells, leading to increased attachment, penetration, and disruption of microbial cell membranes.

3 Antibacterial Mechanism of Mono/Bi-/Tri-metallic Nanoparticles

Metal nanoparticles interact with bacterial cell membrane through several following mechanisms:

3.1 Role of Physiochemical Properties

The physiochemical properties of metal nanoparticles play a crucial role in enhancing antibacterial activities by influencing factors such as drug penetration, size, shape, composition, binding affinity, surface charge, and cellular interactions. The size and shape of metal nanoparticles play crucial role. Nanoparticles with smaller sizes often exhibit increased surface area, allowing more effective contact with bacteria surface area. This can enhance the disruption of cell membranes and increase the cellular update [100]. Moreover, the shape of the metal nanoparticles can impact their ability to adhere to bacterial membrane's and affect internalization processes. Specific shapes may have better binding sites, optimizing interactions with bacteria cells. Optimizing the size and shape of nanoparticles can enhance their their efficacy by improving their ability to interact with bacterial surfaces, penetrate cells, and disrupt essential cellular processes.

3.2 Interaction with Bacterial Cell Membranes

Metal nanoparticles can physically interact with bacterial cell membranes, leading to membrane structural disruption and increased permeability. The interaction can occur through physical contact and electrostatic interactions between the nanoparticles and the bacterial membranes. The disruption of the membrane integrity can result in cell death and inhibition of bacterial growth [101]. The four most prominent routes of antibacterial action of metal nanoparticles are shown in Fig. 2. (1) The MNPs adhere to bacteria cell surface and results in membrane damage and altered transport activity; (2) MNPs diffuse inside the microbial cells and interact with cellular biomolecules, and thereby, affect respective cellular machinery; (3) MNPs causes an increase in ROS inside the microbial cells leading to cell damage and (4) MNPs modulate cellular signal system ultimately causing cell death.

3.3 Generation of Reactive Oxygen Species (ROS)

Metal nanoparticles can enter the bacteria cell, where they may induce oxidative stress by generating reactive oxygen species (ROS) within bacterial cells. ROS, can damage proteins, lipids, and DNA, disrupting vital cellular functions and contributing to antibacterial effects. The excessive

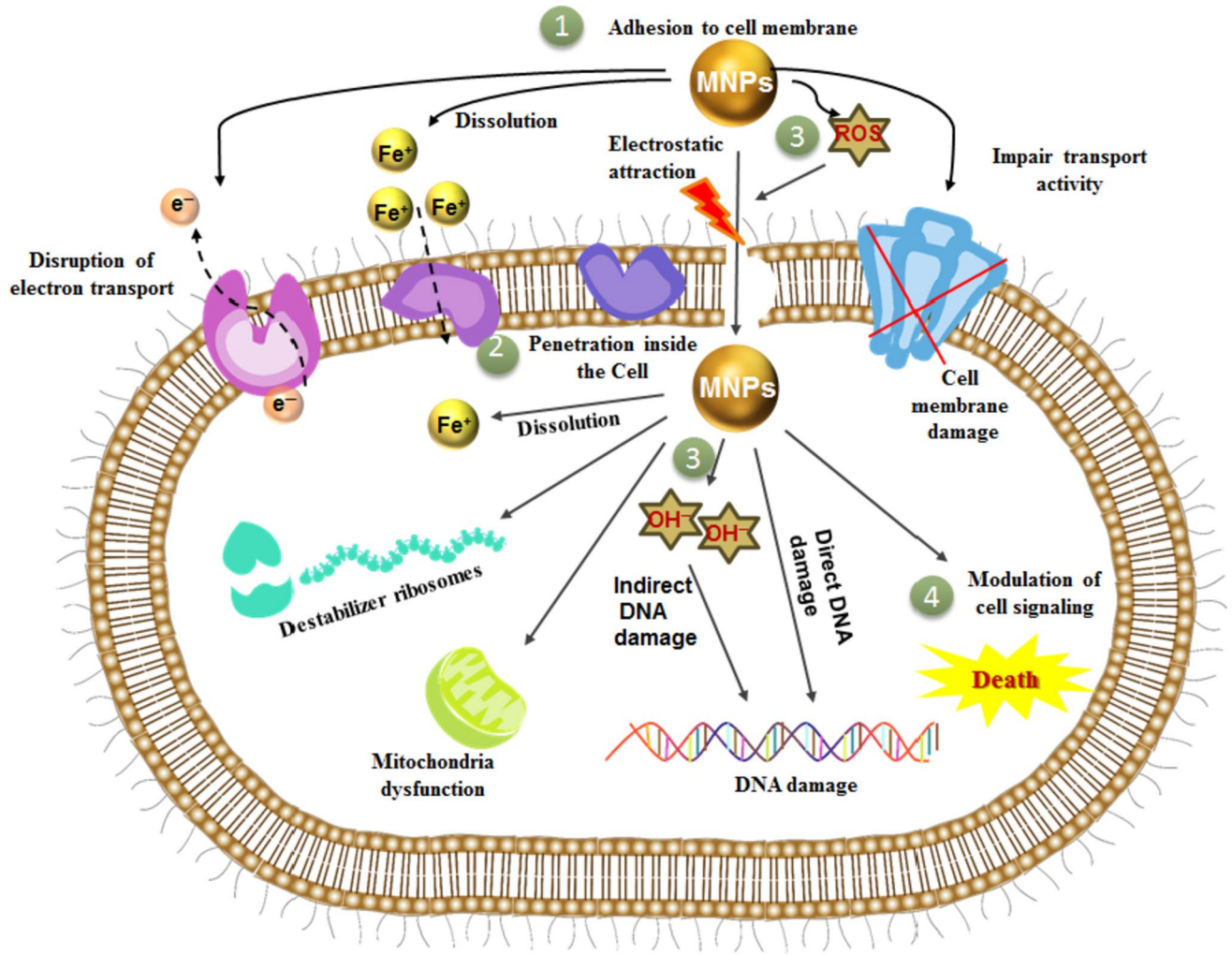

Fig. 2 Mechanism of antibacterial action of metal nanoparticles on a bacteria cell membrane

production of ROS overwhelms the antioxidant defense mechanisms of bacteria, leading to cell death [103].

3.4 Protein Dysfunction and Enzyme Inhibition

Metal nanoparticles can interact with bacterial proteins, leading to protein dysfunction and enzyme inhibition. The binding of nanoparticles to bacterial enzymes can disrupt their structure and function, hindering vital cellular processes. This enzymatic inhibition can compromise the bacterial activity to proliferate and cause infection [103]. This also can impair bacterial metabolism, energy production, and other essential enzymatic activities.

3.5 DNA Damage and Geno-Toxicity

Metal nanoparticles can cause DNA damage in bacterial cells, leading to geno-toxic effects. The nanoparticles can interact with the bacterial DNA, inducing strand breaks, cross-linking, and other forms of damage in bacteria. This disruption of the bacterial DNA integrity can inhibit replication and transcription, ultimately leading to cell death.

Overall, the interaction involves a combination of physical disruption, oxidative stress, and enzyme inhibition, collectively contributing to the antibacterial properties of metal nanoparticles against bacteria [103].

4 Anti-Bacterial Properties of Multi-metallic Nanoparticles (MMNPs)

4.1 Mono-metallic Nanoparticles

Various monometallic nanoparticles have been extensively studied and the results demonstrated potential antibacterial activity against a wide range of bacteria, including antibiotic-resistant strains.

The following are a few examples of well-studied mono-metallic nanoparticles, which show promising antimicrobial activities. Monometallic nanoparticles are made of a single metal element. Some examples of monometallic nanoparticles are silver (Ag), gold (Au), copper (Cu), zinc (Zn), iron (Fe), etc. These metal nanoparticles have been widely studied for their antibacterial or antimicrobial properties against various types of bacteria or microorganisms shown in Tables 1 and 2.

Among the monometallic nanoparticles, silver nanoparticles are the most extensively researched and applied for antibacterial or antimicrobial purposes. Silver nanoparticles have been shown to have a broad-spectrum of antibacterial or antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, viruses, protozoa. Silver nanoparticles can interact with the bacterial cell wall membrane and penetrate the cytoplasm of the nucleus. They can generate reactive oxygen species (ROS) that cause oxidative stress and damage to the bacterial DNA and proteins. They can also interfere with the bacterial metabolism and enzyme activity (Fig. 2).

Other monometallic nanoparticles such as gold, copper, zinc, iron, etc., also have antibacterial or antimicrobial properties against different bacteria or microorganisms. However, their activity is usually lower than that of silver nanoparticles. They may also have some drawbacks such as toxicity to human cells or environmental issues. Therefore, researchers have tried to improve their antibacterial or antimicrobial performance by combining them with other metals to form bimetallic, tri-metallic or multimetallic nanoparticles.

4.1.1 Silver Nanoparticles

Silver nanoparticles (AgNPs) exhibit excellent antimicrobial properties due to their plasmonic ability to disrupt bacterial cell membranes through generation of reactive oxygen species (ROS), which cause protein and DNA damage inside the microbial cells. AgNPs have been reported to be potential candidates against both Gram-positive and Gram-negative bacteria.

4.1.2 Copper Nanoparticles

Copper nanoparticles (CuNPs) possess potent antibacterial properties due to the release of copper ions, which can disrupt bacterial cell membranes, generate reactive oxygen species (ROS), and cause DNA damage. CuNPs exhibit antibacterial efficacy against various bacterial pathogens, including drug-resistant strains.

4.1.3 Gold Nanoparticles

Gold nanoparticles (AuNPs) have been demonstrated as antibacterial activity against both Gram-positive and Gram-negative bacteria. The mechanisms of antibacterial action include membrane disruption, oxidative stress generation, and protein inactivation. However, the antibacterial efficacy of AuNPs may vary depending on the bacterial strain and nanoparticle characteristics.

4.1.4 Zinc Nanoparticles

Zinc nanoparticles (ZnNPs) exhibit antibacterial activity through the release of zinc ions, which disrupt bacterial cellular processes and induce oxidative stress. ZnNPs have shown antibacterial efficacy against a range of pathogens, including antibiotic-resistant strains, making them promising candidates for antimicrobial applications.

4.1.5 Titanium Nanoparticles

Titanium nanoparticles (TiNPs) possess antibacterial activity against various bacterial strains, including drug-resistant pathogens. The antibacterial mechanisms of TiNPs involve membrane disruption, ROS generation, and interference with bacterial cell functions. TiNPs have shown potential in combating bacterial infections and biofilm formation.

The antibacterial activity of different monometallic nanoparticles for two different bacteria pathogen strains E.coli and S.aureus has shown in Fig. 3. The antibacterial activity has shown higher efficacy for silver nanoparticles against *E. coli* bacteria pathogen compared to *S. aureu*s bacteria pathogen. In general, most of the mono metallic nanoparticles show higher antibacterial activity against *E. coli* bacteria pathogen (Gram negative) compared to *S. aureus* (Gram positive) pathogen. From the Table 1, Fig. 1, It is observed that silver and zinc nanoparticles showed higher potential antibacterial activity over other. This increased antibacterial activity *E. coli* compared to *S. aureus* could be attributed to the specific characteristics of the mono-metallic nanoparticles and their interaction with Gram-negative bacteria. It might also involve factors such as cell membrane structure, surface charge that make *E. coli* more susceptible to the antibacterial activity of mono-metallic nanoparticles. The distinctive antibacterial properties of silver and zinc nanoparticles, such as their ability to release ions and affect bacterial cell functions, contribute to their heightened antibacterial activity compared to the other nanoparticles.

4.2 Bi-metallic Nanoparticles

Bimetallic NPs, which are synthesized by combining two different metal salts with tuned size, shape, and structure

Table 1 Antimicrobial activity of Mono MNPs

NPs	Size (nm)	Bacteria	Mode of action	Synthesis	References
Ag	10	*V. natriegens*	DNA damage and cell membrane rupture by reactive oxygen species (ROS)	Green catalysis	[33]
Au	20	*S. pneumoniae*	Cell lysis	Chemical reduction	[34]
Pd	13–18	*S. aureus*, *S. pyogens*, *B. subtilis*	Cell membrane destruction and apoptosis	Biosynthesis (plant)	[35]
Ga	30	*M. tuberculosis*	Reduction of the growth of mycobacterium	Homogenizer	[36]
Cu	15–25	*S. aureus, B. subtilis*	Synergistic effects of organic functional groups	Biosynthesis	[37]
Pt	2–5	*E. coli, A. hydrophilia*	Decrease in bacterial cell viability and Ros generation	chemical reduction	[38]
Si	90–100	*S. aureus, P. aeruginosa*	Mechanical damage of bacteria membrane	laser ablation	[39]
Se	117 55.9 85	*Klebsiella* *B. Subtilis, E. coli* *E. coli, S. aureus*	Production of ROS, disruption of the phospholipid bilayer Ionic interaction between NPs and bacteria caused cell damage Cell membrane due to action of ROS	Biosynthesis (plant) Bio-synthesis Laser Ablation	[40] [41] [42]
Ni	60	*P. aeruginosa*	cell membrane destruction	Bio-synthesis	[43]
Mn	50–100	*S. aureus, E. coli*	inactivation of proteins and decrease in the membrane permeability	Biosynthesis	[44]
Fe	474	*E. coli*	Attraction between negatively charged cell membrane and Nps	Biosynthesis	[45]
Bi	40	*B. anthracis, C. jejuni, E. coli, M. arginini*	Inhibition of proton synthesis	chemical condensation	[46]
Ag_2O	10–60	*S. mutans, L. acidophilus*	Penetration of the cells and hindrance of the growth of pathogen	Biosynthesis	[47]
CuO	60	*B. cereus*	Disturbance of various biochemical process when copper ion invades inside the cell	Biosynthesis	[48]
ZnO	30	*A. baumannii*	Increase in the production of ROS	sol–gel and biosynthesis	[49]
TiO2	9.2	*E. coli*	Decomposition of outer cell and hindrance of the growth of the pathogen	Biosynthesis	[50]
NiO	40–100	*E. coli*	Decomposition of outer cell membrane by Ros,primary Hydroxyl radicals(oH-)	Biosynthesis	[51]
Fe_3O_4	25–40	*S. aureus, E. coli, S. dysentery*	cellular enzyme deactivation and disruption in plasma membrane permeability	Coprecipitation	[52]
CaO	58	*E. coli, S. aureus, K. pneumonia*	cell membrane destruction	Biosynthesis	[53]
MgO	27	*Bacillus* sp., *E. coli*	Destruction of cell membrane integrity resulting in leakage of intercellular materials	Ultrasonication	[54]
CeO_2	5–20	*L. monocytogenes, E. coli, B. cereus*	Ros generation by ceo2 as pro-oxidant	Precipitation	[55]
Mn_3O_4	130	*K. pneumoniae, P. aeruginosa*	membrane damage of bacterial cells by the easy penetration of Mno3o4 Nps	Hydrothermal	[56]
ZrO_2	2.5	*S. mutans, S. mitis, R. dentocariosa, R. mucilaginosa*	Enchantment of the interactions between nps and bacterial constituents	solvothermal	[57]
Ag_2S	65	*Phormidium* spp	inhibition of cell membrane by Ag2s resulting in harmful effects on other biological activities	chemical reduction	[58]
ZnS	65	*Streptococcus* sp, *S. aureus, Lactobacillus* sp, *C. albicans*	Dischargement of ions which react with thiol groups in the proteins present on the cell membrane	Biosynthesis(Bacteria)	[59]
CdS	25	*Streptococcus* sp, *S. aureus, Lactobacillus* sp, *C. albicans*	Impregnation and surrounding the bacterial cells by Cds Nps	Biosynthesis (Bacteria)	[59]

Table 1 (continued)

NPs	Size (nm)	Bacteria	Mode of action	Synthesis	References
FeS	35	*S. aureus, E. coli, E. faecalis*	Np internalization through fine cell membrane	Hydrothermal	[60]
Mn-MOF	–	*E. coli, E. faecalis, S. aureus, P. aeruginosa*	Peptide–nalidixic acid conjugate formation	Mechanochemical	[61]
Mg-MOF	–	*E. coli, E. faecalis, S. aureus, P. aeruginosa*	Peptide–nalidixic acid conjugate formation	Mechanochemical	[61]
Ag-MOF	–	*S. aureus*	High stability in water and the existence of Ag ion	solvothermal	[62]
Cu-MOF	–	*S. aureus, E. coli, K. pneumonia, P. aeruginosa, S. aureus*	Attachment to the bacterial surfaces by active surface metal sites in Cu-MOF	Hydrothermal	[63]
Zn-MOF	–	*P. aeruginosa*	Penetration inside the bacteria, causing cell damage by interaction with lipotropic acid	Solvothermal	[64]
Co-MOF	–	*E. coli*	Strong interaction with membranes containing glycerophosphoryl moieties	Hydro-solvothermal	[65]

Table 2 Antimicrobial activity of Mono MNPs

S. no.	Name of the particles	Zone of inhibition (mm)		References
		E. coli	*S. aureus*	
1	Ag	13.9 ± 0.8	11.6 ± 1.1	[66]
2	Ag	22	15	[67]
4	Au	13	17	[68]
5	Au	17.9	17.3	[69]
6	Cu	10.8	12.6	[70]
7	Cu	18	24	[71]
8	Zn	24	16	[71]
9	Zn	23	14	[71]

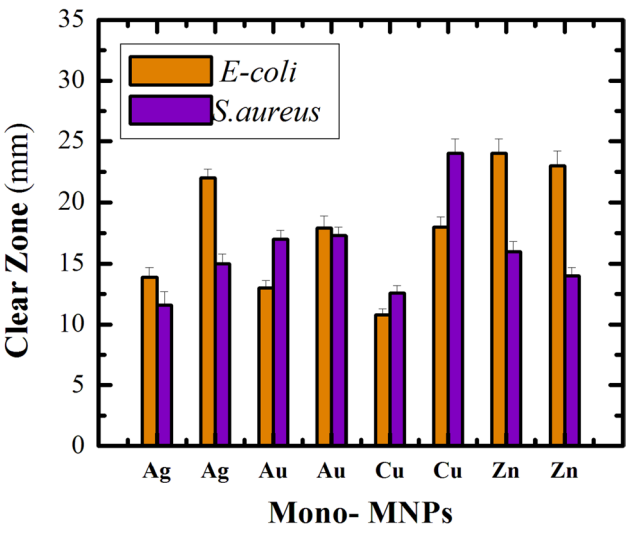

Fig. 3 Antibacterial activity of Monometallic NPs against two bacteria pathogen strains: *S. aureus* & *E. coli* using a disk diffusion method (clear zone)

[31–33, 41, 42, 72–76] have attracted more attention due to their modified multi-functional properties. Silver-copper nanoparticles (Ag-CuNPs) combine the potent antibacterial properties of both metals [34]. The synergistic effects of silver (Ag) and copper (Cu) in Ag–Cu NPs lead to increased ROS generation, disruption of cell membranes, and DNA damage, resulting in enhanced antibacterial activity against a broad spectrum of bacterial strains, including drug-resistant pathogens [35]. Silver-gold nanoparticles (Ag-AuNPs) demonstrate synergistic antibacterial effects by combining the membrane-damaging properties of silver with the oxidative stress-inducing properties of gold. Depending on their physicochemical properties of two metals and their spatial overlapping distribution can lead to the formation of a nano-core shell structure due to impact of individual metals properties. The addition of second dopant metal can help to tune electronic structure of NPs, which enhance or increase the multifunctional properties (antibacterial activity, catalytic properties) of the bi-metallic NPs.

4.3 Antibacterial Activities of Bimetallic Nanoparticles

Bimetallic nanoparticles are nanoparticles composed of two different metals. They can have different structures such as alloys (homogeneous mixing of two metals), core–shell (one metal forms a core surrounded by another metal shell) or contact aggregates (two metals form separate domains in contact with each other). Bimetallic nanoparticles can exhibit new and improved properties compared to their monometallic counterparts due to the synergistic effects between the two metals. Bimetallic nanoparticles have attracted a lot of attention for their antibacterial or antimicrobial applications because they can enhance the activity and selectivity

of the constituent metals. They can also reduce the cost and toxicity of the noble metals by using less amount or by alloying with cheaper metals. The antibacterial or antimicrobial mechanisms of bimetallic nanoparticles depend on their structure and composition. For example, alloy bimetallic nanoparticles can have enhanced electronic properties that affect their interaction with the bacterial cells or their ROS generation. Core–shell bimetallic nanoparticles can improve stability and durability of the core metal by the protection of the shell metal. Contact aggregate bimetallic nanoparticles can have increased surface area and reactivity due to the presence of more interfaces between the two metals.

Bimetallic nanoparticles, due to the synergistic effects of two different metals, result in enhanced antibacterial properties compared to monometallic counterparts. For example, silver-copper nanoparticles (Ag–Cu NPs) and silver-gold nanoparticles (Ag–Au NPs) have shown synergistic effects, combining the strength of both metals. The enhanced stability and controlled release of metal ions by bimetallic nanoparticles (BMNPs) show a broader spectrum of activity against a range of bacterial strains. The antibacterial activity of different bi-metallic nanoparticles has been reported in various articles and shown in Table 3 **&** Table 4.

Table 4 Antimicrobial activity of Bi- MNPs

S. no.	Name of the particles	zone of inhibition (mm)		References
		E. coli	*S. aureus*	
1	Au–Pt	15	13	[88]
2	Ag–Au	20	22	[89]
3	Au–Zno	11	8	[90]
4	Ag–Co	22	–	[91]
5	Mo–Zno	7.5	–	[92]
6	Mn–Cu	16.3.9 ± 0.57	14.33 ± 0.57	[93]

4.3.1 Silver-Copper Nanoparticles

Bimetallic nanoparticles composed of silver and copper (Ag–Cu NPs) have shown enhanced antibacterial activities compared to their monometallic counterparts. The combination of silver and copper ions can lead to synergistic effects,

Table 3 Antimicrobial activity of bimetallic NPs

NPs	Size (nm)	Bacteria	Mode of action	Synthesis	References
Ag/Au	9.7	E	Increased production of ROS	Green	[36]
Ag/Cu	26	*E. coli, B. subtilis*	Permeability of copper and silver ions into the bacterial cell membrane	Biosynthesis (plant)	[36]
Au/Pt	2–10	*S. aureus, P. aeruginosa, C. albicans*	Release of Ag + ions, unbalance of cell metabolism, and ROS generation	Chemical reduction	[37]
Ag/Fe	110	*S. aureus, P. aeruginosa*	Release of Ag + ions and ROS generation	Electrical explosion	[38]
Ag/Pt	36	*E. faecalis, E. coli*	Increased production of ROS	Biosynthesis	[39]
Cu/Zn	100	*A. faecalis, S. aureus, C. freundii*	Synergistic properties of Zn2 + and Cu2 + ions together	Biosynthesis	[40]
Cu-Ni	25	*S. mutans, S. aureus, E. coli*	Strong adsorption of ions to the bacterial cells	Chemical [90] reduction	[77]
Ag/ZnO	43	*S. aureus, P. aeruginosa*	Ag + leaching from metallic silver	Photoreduction	[78]
Ag/SnO_2	9	*B. subtilis, P. aeruginosa, E. coli*	Synergistic properties of Ag and SnO	Biosynthesis [92] (plant)	[79]
Cu/FeO_2	32.4	*B. subtilis, X. campestris*	DNA damage induced by NPs	Hydrothermal	[80]
Au/CuS	2–5	*B. anthracis*	Disordered and damaged membranes	Seeded	[81]
Fe_3S_4/Ag	226	*S. aureus, E. coli*	Release of Ag + ions and ROS generation	Solvothermal	[82]
MgO/ZnO	10	*P. mirabilis*	Alteration of cell membrane activity, ion release, and ROS production	Precipitation	[83]
CuO/ZnO	50 and 82	*E. coli, S. aureus*	Electrostatic interaction causing to change membrane permeability on account of depolarization	electrical explosion	[84]
CuO/Ag	20–100	*L. innocua, S. enteritidis*	Binding of the ions released by μCuO/nAg to the thiol groups of many enzymes in cell membrane	Hydrothermal	[85]
Fe_3O4/Zn0	200–800	*S. aureus, E. coli*	Membrane stress, disrupting and damaging cell membrane	Co-precipitation	[86]
CeO_2/FeO_2	40 and 25	*P. aeruginosa*	Combination of NPs with antibiotic ciprofloxacin, causing inhibitory effect on bacterial growth and biofilm formation	Hydrothermal	[87]

resulting in increased disruption of bacterial membranes, oxidative stress generation, and inhibition of bacterial growth. Ag–Cu NPs have demonstrated potent antibacterial efficacy against various pathogens, including drug-resistant bacteria.

4.3.2 Silver-Gold Nanoparticles

Bimetallic nanoparticles consisting of silver and gold (Ag–Au NPs) exhibit synergistic antibacterial effects. Ag–Au NPs have demonstrated enhanced antibacterial activity compared to monometallic nanoparticles made of the individual metal alone. The enhanced antimicrobial performance is attributed to the co-operative action of silver and gold ions. The antibacterial mechanisms of Ag–Au NPs involve membrane disruption, generation of reactive oxygen species (ROS), and interference with bacterial cellular functions.

4.3.3 Copper–Zinc Nanoparticles

Bimetallic nanoparticles comprising copper and zinc (Cu–Zn NPs) exhibit strong antibacterial properties. The combination of copper and zinc ions synergistically disrupts bacterial membranes, inhibits enzymatic processes, and induces oxidative stress, leading to effective bacterial cell damage. Cu–Zn NPs have demonstrated significant antibacterial efficacy against various bacterial strains.

4.3.4 Gold–Zinc Nanoparticles

Bimetallic nanoparticles composed of gold and zinc (Au–Zn NPs) possess antibacterial properties that arise from the synergistic effects of gold and zinc ions. Au–Zn NPs have shown effective antibacterial activity against various bacterial pathogens, including drug-resistant strains. The antibacterial mechanisms involve membrane disruption, ROS generation, and interference with bacterial cellular functions.

The antibacterial activity of different bimetallic nanoparticles with *E. coli* and *S. aureus* pathogens has been shown in Fig. 4. The antibacterial activity is reported as higher efficacy for silver–gold nanoparticles against both *E. coli* and *S. aureus* bacteria pathogens. In general, most of the bimetallic nanoparticles observed higher antibacterial activity against *E. coli* bacteria pathogen (Gram negative) compared to *S. aureus* (Gram positive) pathogen which might be due to more electrostatic surface charge interactions between MNPs and bacteria cell membrane. Bimetallic nanoparticles, due to the synergistic effects of two different metals, result in enhanced antibacterial properties against monometallic antibacterial properties.

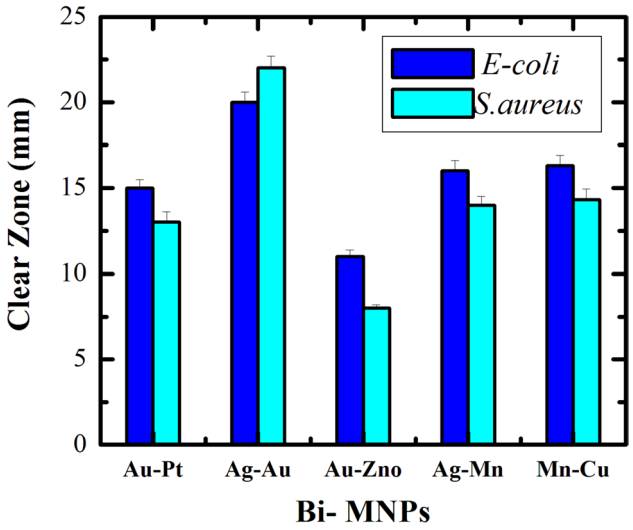

Fig. 4 Antibacterial activity of Bi-metallic NPs against two bacteria pathogen strains: *S. aureus* & *E. coli* using a disk diffusion method

4.4 Tri-metallic Nanoparticles

Tri-metallic nanoparticles are nanoparticles composed of three different metals. They can have different structures such as alloys, core-shells, or contact aggregates. Tri-metallic nanoparticles can exhibit more complex and diverse properties than bimetallic nanoparticles due to the additional interactions among the three metals.

Tri-metallic nanoparticles have been explored for their antibacterial applications because they can further improve the activity and selectivity of the constituent metals. They can also reduce the cost and toxicity of the noble metals by using fewer amounts or by alloying with cheaper metals. Some examples of tri-metallic nanoparticles are Ag–Au–Pt, Ag–Au–Cu, Ag–Cu–ZnO, Au–Pt–Pd, Cu–ZnO–TiO_2 Cu–ZnO–TiO2, etc.

The antibacterial mechanisms of tri-metallic nanoparticles depend on their structure and composition. For example, alloy tri-metallic nanoparticles can have enhanced electronic properties that affect their interaction with the bacterial cells or their ROS generation. Core–shell tri-metallic nanoparticles can improve stability and durability of the core metal by the protection of the shell metal. Contact aggregate tri-metallic nanoparticles can have increased surface area and reactivity due to the presence of more interfaces among the three metals. The antibacterial activity of tri-metallic nanoparticles have been reported in literature and shown in Tables 5 and 6.

4.4.1 Silver–Copper–Zinc Nanoparticles

As the name suggests, tri-metallic nanoparticles are composed of the combination of three different metals, offering

Table 5 Antimicrobial activity of trimetallic NPs

NPs	Size (nm)	Bacteria	Mode of action	Synthesis	References
Cu/Zn/Fe	42	*E. faecalis, E. coli*	Interruption of cellular processes by released ions, which can cross cell membranes	Chemical reduction	[94]
Au/Pt/Ag	20–40	*E. coli, S. typhi, E. faecalis*	Generation of ROS	Microwave	[95]
Cu/Cr/Ni	100–200	*E. coli, S. aureus*	Antibacterial activity of trimetallic NPs in comparison with pure metals	Biosynthesis [63] (plant)	[80]
CuO/NiO/ZnO	7	*S. aureus, E. coli*	Ruptured and cracked bacterial cells by the release of intracellular components	Coprecipitation	[96]
Ag/ZnO/TiO_2	60–170	*E. coli*	Reduction in the bandgap energy by increasing the e − & h + charge separation tim	Sol–gel	[97]

Table 6 Antimicrobial activity of Tri- MNPs

S. no.	Name of the particles	zone of inhibition (mm)		References
		E. coli	*S. aureus*	
1	Ag/Au/Pt	24	22	[98]
3	Ag/Au/Zn	12	13	[99]

further opportunities for enhanced antibacterial activity. While studies on antimicrobial activities of tri-metallic nanoparticles are relatively limited, there are potential advantages to explore the antimicrobial performance of these NPs. Synergistic effects of three metals may result in even greater antibacterial activity through a wider range of mechanisms of action, for potentially targeting specific bacterial strains or antibiotic-resistant pathogens.

4.4.2 Silver–Gold–Copper Nanoparticles

Tri-metallic nanoparticles composed of silver, gold, and copper (Ag–Au–Cu NPs) exhibit enhanced antibacterial activities compared to their individual mono/bi-metallic counterparts. The synergistic combination of these metals enhances the disruption of bacterial cell membranes, ROS generation, and interference with bacterial cellular processes, leading to effective antibacterial action. Ag–Au–Cu NPs have shown promising antibacterial efficacy against various bacterial strains.

4.4.3 Gold–Copper–Zinc Nanoparticles

Tri-metallic nanoparticles combining gold, copper, and zinc (Au–Cu–Zn NPs) have shown enhanced antibacterial activities compared to the nanoparticles formed with each individual component metal. The combination of these three metals provides a synergistic effect, leading to increased disruption of bacterial membranes, ROS generation, and interference with bacterial cellular processes. Au–Cu–Zn NPs have demonstrated effective antibacterial efficacy against various bacterial strains.

4.5 Antibacterial Activities of Multimetallic Nanoparticles

Multimetallic nanoparticles are nanoparticles composed of more than three different metals. They can have different structures such as alloys, core-shells, or contact aggregates. Multimetallic nanoparticles can exhibit more complex and diverse properties than trimetallic nanoparticles due to the additional interactions among the multiple metals.

Multimetallic nanoparticles have been explored for their antibacterial or antimicrobial applications because they can further improve the activity and selectivity of the constituent metals. They can also reduce the cost and toxicity of the noble metals by using less amount or by alloying with cheaper metals. Some examples of multimetallic nanoparticles are Ag–Au–Cu–Pt, Ag–Au–Cu–ZnO, Ag–Au–Pt–Pd, Au–Pt–Pd–Rh, Cu–ZnO–TiO2, Cu–ZnO–TiO2, Cu–ZnO–Fe_3O_4, Cu–ZnO–Fe_3O_4, etc.

The antibacterial or antimicrobial mechanisms of multimetallic nanoparticles depend on their structure and composition. For example, alloy multimetallic nanoparticles can have enhanced electronic properties that affect their interaction with the bacterial cells or their ROS generation. Core–shell multimetallic nanoparticles can have improved stability and durability of the core metal by the protection of the shell metal. Contact aggregate multimetallic nanoparticles can have increased surface area and reactivity due to the presence of more interfaces among the multiple metals.

Multimetallic nanoparticles (MMNPs) are composed of three or more different metal elements. The composition and synthesis methods of MMNPs greatly influence their antibacterial activities. The selection of metals and their ratios in MMNPs can be tailored to achieve desired properties and optimize antibacterial efficacy. Various synthesis methods such as co-reduction, sequential reduction, and galvanic replacement have been employed to prepare MMNPs with controlled compositions and structures.

4.5.1 Enhanced Antibacterial Activities and Synergistic Effects

Multimetallic nanoparticles often exhibit enhanced antibacterial activities compared to monometallic, bimetallic and tri-metallic counterpart nanoparticles. The combination of multiple metals in MMNPs leads to synergistic effects, in which case the antibacterial efficacy is greater than the sum of individual metallic components. The synergistic effects arise from cooperative interactions between metals, which result in increased disruption of bacterial membranes, generation of reactive oxygen species (ROS), and interference with bacterial cellular functions.

4.5.2 Multimetallic Nanoparticles and Their Antibacterial Mechanisms

The antibacterial activity of various tri-metallic nanoparticles for *E. coli* and *S. aureus* pathogens has shown in Fig. 5. The antibacterial activity is reported as higher efficacy for silver–gold–platinum nanoparticles against *E. coli* bacteria pathogens. In general, most of the bi- metallic nanoparticles observed higher antibacterial activity against *E. coli* bacteria pathogen (Gram negative) compared to *S. aureus* (Gram positive) pathogen which might be due to more electrostatic surface charge interactions between tri-MNPs and bacteria cell membrane. Tri-metallic nanoparticles, due to the synergistic effects of two different metals, result in enhanced antibacterial properties against bi/monometallic antibacterial properties. The antibacterial activity of all mono-bi-tri metallic nanoparticles is shown Fig. 6.The synergistic effects arise from cooperative interactions between three different metals, which result in enhanced disruption of bacterial membrane

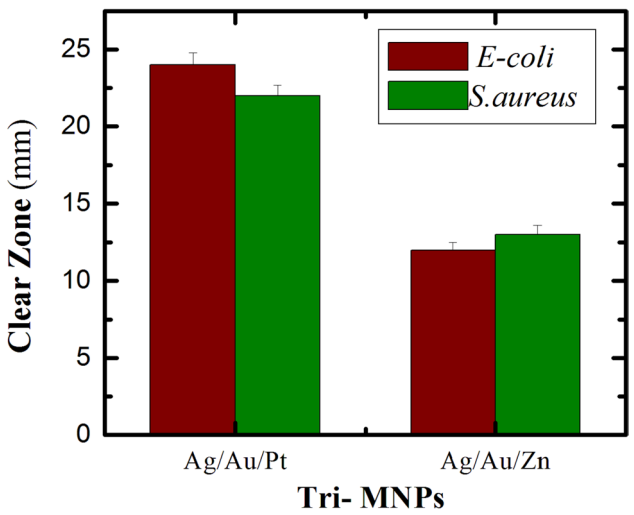

Fig. 5 Antibacterial activity of Tri-metallic NPs against the bacteria pathogens of *S. aureus* & *E. coli*

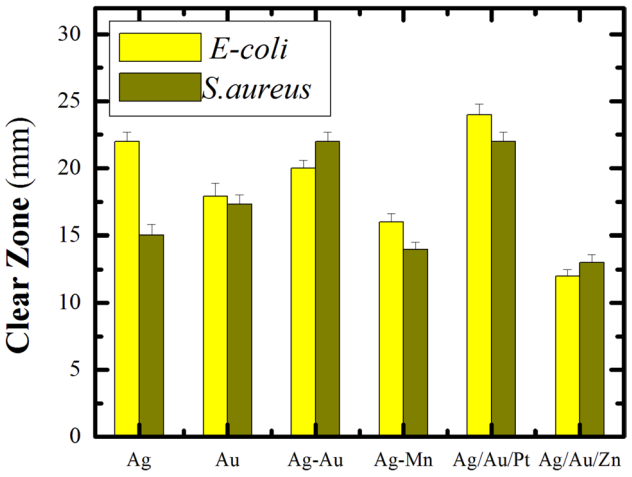

Fig. 6 Antibacterial activity of MNPs against the bacteria pathogens of *S. aureus* & *E. coli* bacteria

cell, generation of reactive oxygen species (ROS), and interference with bacterial cellular functions.

5 Synergistic Effect Metallic Nanoparticles for Antibacterial Activity

Selection of types of metal nanoparticles among monometallic, bimetallic, and trimetallic nanoparticles are most challenging in terms of antibacterial activity. It is not straightforward process and it depends on several factors. There are potential advantages of each type of MNPs. The choice of the "best" type of MNP for antibacterial activity depends on the specific application, target bacteria, and desired outcomes. Monometallic nanoparticles like AgNPs are well established and widely used, but synergistic effects of bimetallic and trimetallic nanoparticles offer the potential for enhanced efficacy and tailored properties. It is important to consider factors such as cost, ease of synthesis, stability, long-term toxicity, and potential resistance development when evaluating the effectiveness of MNPs as antibacterial agents. Further research is required to explore the potential antibacterial activity of bi-metallic and tri-metallic nanoparticles, optimize their synthesis methods, understand their mechanisms of action, and evaluate their long-term safety and efficacy for various bacterial infections.

6 Concluding Remarks and Future Directions

The antibacterial properties of MNPs hold great promise for combating bacterial infections, including multidrug-resistant strains. The unique physiochemical properties of MNPs,

coupled with their ability to disrupt bacterial cell membranes, generate reactive oxygen species, induce protein dysfunction, and cause DNA damage, contribute to their antibacterial mechanisms. The comparative analysis of antibacterial activities among monometallic, bimetallic, tri-metallic, and multimetallic nanoparticles demonstrates the potential of these nanomaterials in combating bacterial infections. The development of targeted antibacterial strategies utilizing MNPs can help overcome the challenges posed by antibiotic-resistant bacteria. Furthermore, MNPs can find applications in various fields, including medicine, biotechnology, and environmental remediation, offering innovative solutions for combating bacterial microbial infections and addressing global health concerns. We have also discussed the factors that influence their antibacterial or antimicrobial performance, such as size, shape, composition, structure, surface modification, and concentration.

We have observed that monometallic nanoparticles such as silver, gold, copper, zinc, iron, etc., have potential antibacterial properties against different bacteria. However, their activity is usually lower than that of bimetallic, tri-metallic or multi-metallic nanoparticles. Bimetallic, tri-metallic or multimetallic nanoparticles can exhibit new and improved properties compared to their monometallic counterparts due to the synergistic effects among the constituent metal components. They can also reduce the cost and toxicity of the noble metals by using fewer amounts or by alloying with cheaper metals. Therefore, bimetallic, tri-metallic or multi-metallic nanoparticles are promising candidates for antibacterial or antimicrobial applications in various fields such as biomedicine, food packaging, and water treatment. However, there are still some challenges and limitations that need to be addressed before their widespread use in practical applications.

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Declarations

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Conflict of Interest The authors have no relevant financial interest to disclose.

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