

REVIEWS

FACTORS GOVERNING THE ANTIBACTERIAL ACTIVITY OF METAL-BASED NANOPARTICLES: PHYSICOCHEMICAL PROPERTIES, BIOLOGICAL ENVIRONMENT, AND EXPERIMENTAL CONDITIONS

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ABSTRACT

INTRODUCTION: The antibacterial activity of metal-based nanoparticles (MBNPs) varies considerably even among materials with the same elemental composition. Differences in particle size, morphology, surface properties, aggregation, synthesis, and exposure conditions can alter nanoparticle–bacteria interactions and complicate comparisons between studies.

AIM: This review explores the principal physicochemical and experimental factors that determine the antibacterial performance of MBNPs and considers how these variables influence the interpretation and reproducibility of antibacterial studies.

MATERIALS AND METHODS: A narrative literature review was conducted using Scopus, Web of Science, and PubMed. Studies addressing the relationship between MBNP characteristics or experimental conditions and antibacterial activity were considered.

RESULTS AND CONCLUSION: Antibacterial performance is determined by a combination of particle-related, biological, and experimental variables rather than nanoparticle composition alone. Smaller particle dimensions can increase available surface area and modify dissolution and surface reactivity, while morphology and surface chemistry influence bacterial contact and interfacial processes. Surface coatings can either restrict or enhance biological interactions, and aggregation may substantially change the effective particle size during antibacterial testing. Concentration, dissolution and metal-ion release, pH, ionic strength, proteins, organic matter, and light exposure further modify nanoparticle behavior. Differences in bacterial strain, growth state, inoculum, and assay methodology introduce additional variability and may contribute to apparently contradictory findings between studies. The antibacterial activity of MBNPs should be interpreted as the outcome of an interacting set of material and experimental variables rather than as a fixed property of a particular metal or metal oxide. Improved physicochemical characterization and more transparent reporting of microbiological test conditions are necessary for meaningful comparison between

studies. Establishing reproducible relationships between nanoparticle characteristics and antibacterial performance will be important for the rational development of MBNP-based antimicrobial systems.

Keywords: aggregation, antibacterial activity, antibacterial testing, metal-based nanoparticles, metal-ion release, nanoparticle functionalization, particle size, surface charge

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1. INTRODUCTION

Metal-based nanoparticles (MBNPs) continue to attract attention as antibacterial materials because their nanoscale dimensions provide physicochemical characteristics that differ from those of the corresponding bulk materials (1–3). Depending on their composition, they may interact with bacterial surfaces, release biologically active metal ions, promote reactive oxygen species (ROS) formation, or interfere with essential cellular processes (1,4,5). Antibacterial activity has consequently been reported for several MBNPs against both Gram-positive and Gram-negative bacteria (2,3,5).

However, antibacterial activity does not imply that nanoparticles of the same chemical composition will necessarily behave the same way (1,5–7). Studies report considerable differences between apparently similar nanomaterials (6,7). A silver nanoparticle, for example, cannot be defined biologically by its silver content alone. Its dimensions, morphology, surface coating, charge, aggregation state, and dissolution behavior all contribute to the form in which the particle encounters a bacterial cell. The same principle applies to other MBNPs (1,5).

The aforementioned variables are primarily governed by the experimental procedure for MBNP synthesis (e.g., choice of reductant, reductant-to-salt ratio, etc.), are also interconnected (8). For example, a decrease in particle size changes the surface-area-to-volume ratio but may simultaneously affect dissolution and aggregation (5–7). A surface coating can improve colloidal stability while limiting direct contact between the inorganic surface and bacterial envelope (6,7). The surrounding medium may alter surface charge, promote aggregation, or lead to adsorption of proteins and other biomolecules. Consequently, a physicochemical characteristic examined in isolation may not fully explain the antibacterial response (5–8).

Variation also originates from the biological and experimental aspects of nanoparticle–bacterium interactions. Bacterial species and strains differ in cell-envelope organization and physiological characteristics, while inoculum density, growth phase, exposure time, culture medium, and antibacterial assay can influence the measured outcome. A nanoparticle preparation that appears highly active under one

set of experimental conditions may therefore show a different response when the test system changes. This becomes particularly important when results obtained in simplified laboratory media are used to predict performance in biological or environmental settings (5,7,9,10).

Understanding the sources of variability matters for more than comparing published studies. Identifying relationships between nanoparticle characteristics and antibacterial performance can support rational material design, while recognizing experimental sources of variation can improve reproducibility. A review focused specifically on these determinants may therefore help distinguish the intrinsic contribution of the nanomaterial from effects introduced by its preparation, the surrounding environment, the bacterial target, or the testing procedure (6–10).

2. AIM

The aim of this review is to examine the key factors governing the antibacterial activity of MBNPs. Particular attention is given to particle size and morphology, surface charge and functionalization, concentration, aggregation and colloidal stability, dissolution and metal-ion release, synthesis method, and environmental conditions. The study also considers biological and methodological variables that influence the measured antibacterial response, with the broader objective of identifying factors to consider when comparing MBNP antibacterial studies and designing future antimicrobial nanoparticle systems.

3. MATERIALS AND METHODS

A narrative literature review was conducted to identify studies examining factors that influence MBNP antibacterial activity. Literature searches were performed in Scopus, Web of Science, and PubMed using combinations of terms related to “metal nanoparticles,” “metal oxide nanoparticles,” “antibacterial activity,” “particle size,” “shape,” “morphology,” “surface charge,” “surface coating,” “functionalization,” “aggregation,” “dissolution,” “metal ion release,” “synthesis,” “culture medium,” and “antibacterial assay.”

Priority was given to peer-reviewed studies published in the last five years (from 2022 to 2026) examining relationships between nanoparticle charac-

teristics or experimental conditions and antibacterial outcomes, together with recent reviews providing broader interpretations of these relationships. Earlier studies of particular relevance to the topics discussed were also considered where appropriate. Studies concerning silver, gold, copper/copper oxide, zinc oxide, titanium dioxide, iron/iron oxide, and related antibacterial nanomaterials were considered when relevant to the factor under discussion. As this work was designed as a narrative rather than a systematic review, study selection was based on relevance to the scope and objectives of the review, and a formal systematic-review screening and study-selection protocol was not applied.

The literature was organized according to particle-related, environmental, biological, and methodological determinants of antibacterial activity. Because the objective was to provide a narrative synthesis rather than a quantitative comparison or me-

ta-analysis, findings were interpreted according to reported trends and mechanistic relationships rather than pooled effect estimates.

AI-assisted technology (ChatGPT OpenAI, GPT-5.5) was used solely for language polishing and figure generation. All scientific content, interpretations, and conclusions were conceived, verified, and approved by the authors.

4. RESULTS

4.1. Factors Affecting Antibacterial Activity

The antibacterial performance of MBNPs cannot be predicted from elemental composition alone. Nanoparticles prepared from the same material may behave differently when their dimensions, morphology, surface properties, or state of dispersion differ. These characteristics influence the surface presented to bacterial cells, the stability of the particles in suspension, their interaction with the surround-

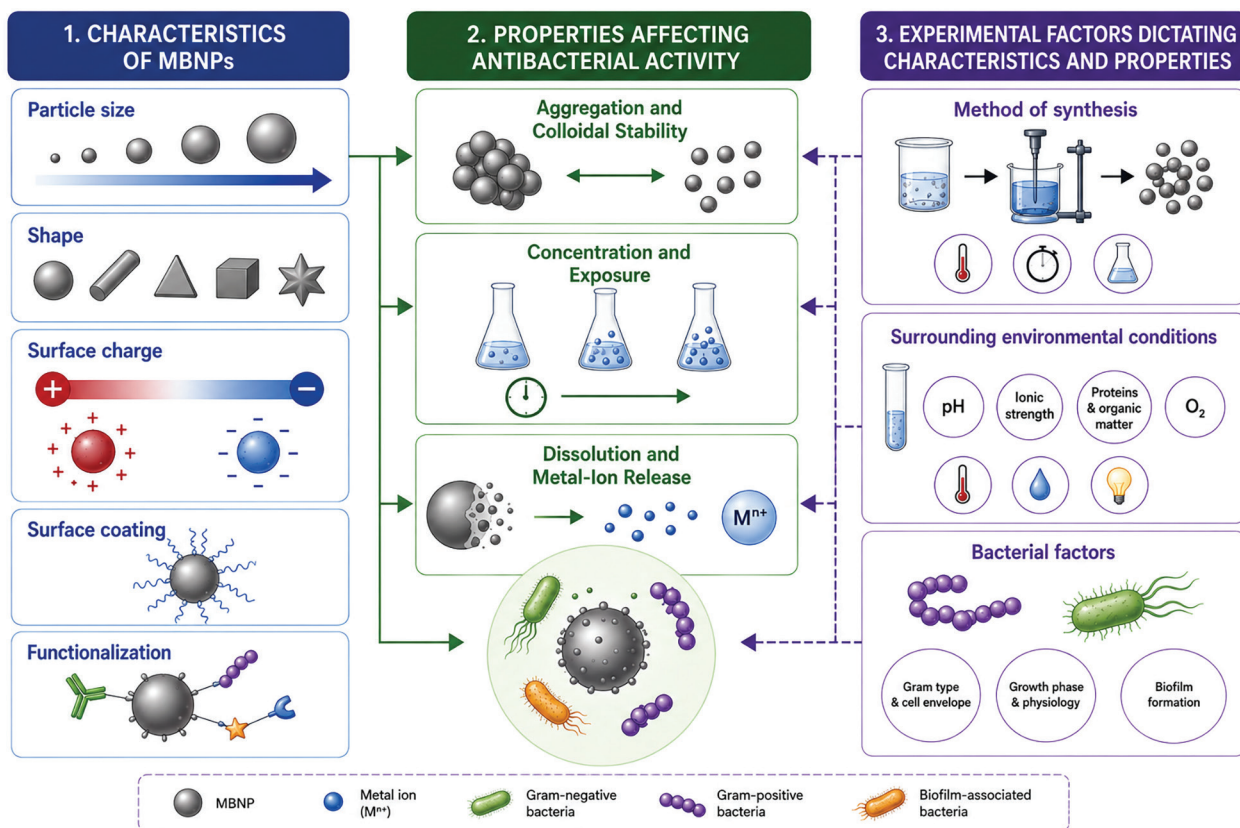


Fig. 1. Factors affecting antibacterial activity of MBNPs—physicochemical characteristics, properties and experimental factors. This figure was created with the assistance of ChatGPT (OpenAI, GPT-5.5). Artificial intelligence was used solely to generate the graphical illustration based on detailed author instructions. The scientific content, figure design, labels, workflow, and all technical information were conceived, verified, edited, and approved by the authors, who assume full responsibility for the final figure.

ing medium, and, where applicable, dissolution and ROS generation. In this respect, key physicochemical characteristics of MBNPs are herein related to processes and circumstances determining their physical state and biological reactivity, and ultimately, to their properties directing the antibacterial activity (1,5–7) (Fig. 1).

4.1.1. Physicochemical Characteristics of MBNPs Affecting Antimicrobial Activity

Particle Size

Particle size strongly affects antibacterial activity because smaller nanoparticles generally provide a higher surface-area-to-volume ratio. Consequently, more atoms are exposed at the particle surface, where interactions with the surrounding medium and bacterial cells occur. Depending on the material, this may favor dissolution and ion release, surface reactions, ROS formation, or direct interaction with the bacterial envelope. Smaller particles may also establish closer or more extensive contact with bacterial surfaces (5–7,9,11).

However, the relationship between size and antibacterial activity is not linear. Very small particles may aggregate, particularly in media containing salts, proteins, or other biological components, thereby reducing the effective surface area available for interaction. Surface coating and dissolution behavior can further modify the size-dependent response. Thus, nominal particle diameter alone is insufficient to predict antibacterial performance (6,7,9,11).

Shape

Nanoparticles can occur as spheres, rods, triangles, plates, cubes, stars, flower-like structures, and numerous other morphologies. Shape affects more than the physical appearance of the particle because different geometries expose different crystallographic facets and alter the proportion of surface atoms available for interaction. As a result, nanoparticles of the same chemical composition but different morphologies may differ in surface reactivity, dissolution, ROS production, and their interactions with bacterial envelopes. This effect is highly material-dependent, and no single morphology can be considered universally superior for antibacterial purposes. However, several studies have demonstrated the stronger antibacterial activity of nanosized cubes, triangles, rods, or wires in comparison to spherical

MBNPs, owing to increased surface-to-volume area, sharp edges that may mechanically damage bacterial cell walls (by triangular plates), and/or geometrically facilitated penetration in bacterial biofilms (in the case of rods and wires). Comparisons of particle shape require caution because altering the synthesis conditions used to control morphology often changes particle size and surface chemistry at the same time (6,12–14).

Surface Charge

Bacterial surfaces are generally negatively charged under physiological conditions. However, this electrostatic attraction is heavily modulated by the ionic strength of the surrounding medium and the specific lipopolysaccharide or teichoic acid composition of the target strain, increasing particle accumulation at the cell surface and potentially facilitating membrane disruption (7,15). Surface charge can also influence colloidal stability and nanoparticle interactions with proteins and other components of biological media (5,7,16).

A strongly positive surface is not necessarily desirable, however (16,17). The same interactions that favor contact with bacteria can increase nonspecific association with mammalian cell membranes and consequently affect cytocompatibility. Surface charge, therefore, needs to be considered alongside antibacterial selectivity rather than antibacterial potency alone (7,14,16,17).

Surface Coating and Functionalization

The surface of MBNPs is rarely chemically inert (6,7,18,19). Stabilizers, polymers, surfactants, biomolecules, or deliberately attached functional groups can change particle charge, hydrophobicity, colloidal stability, and interaction with biological surfaces (6,7,16,19). A coating may reduce aggregation and improve dispersion, but it can also restrict dissolution or physically separate the inorganic surface from the bacterial envelope. Conversely, functionalization with antibiotics, antimicrobial peptides, or bacterial-targeting ligands may enhance antibacterial activity or improve selectivity (6,7,18,19). For this reason, results obtained with coated or functionalized nanoparticles should not automatically be attributed to the metal or metal oxide core. The biological response reflects the properties of the complete nanoparticle system (6,7,16,19).

4.1.2. Properties of MBNPs Dependent on the Physicochemical Characteristics and Affecting Antimicrobial Activity

Aggregation and Colloidal Stability

Aggregation is closely connected to several of the characteristics described above. Nanoparticles characterized in water or immediately after synthesis may behave quite differently once introduced into culture medium, physiological fluids, or environmental samples. Increased ionic strength can screen surface charges and promote aggregation, while proteins and other biomolecules may adsorb onto the particle surface, forming a corona. Such changes alter the effective particle size and the surface presented to bacterial cells (7,9,16). Moreover, such events may complicate the process of determining the minimum inhibitory concentrations (MICs) using turbidity-based detection. For example, there have been documented cases of colloidal destruction and palpable opalescence when MBNP samples are introduced into inoculated bacterial culture media (20).

Concentration and Exposure

Nanoparticle concentration is one of the most immediate determinants of the antibacterial response (1,7,9,20). Increasing concentration generally increases the number of particles available for interaction with bacterial cells and, for dissolving materials, may increase the amount of biologically available metal ions; however, higher concentrations may simultaneously promote aggregation in high-ionic-strength media, potentially reducing the effective surface area available (1,9). Dose-dependent reductions in bacterial growth or viability are therefore commonly reported (1,7,20). The relationship is not necessarily proportional, however, because changes in aggregation, sedimentation, dissolution, and surface availability can occur as concentration increases (7,9,20).

Exposure time should be considered together with concentration. A high concentration applied for a short period and a lower concentration maintained for longer may not produce equivalent effects (1,5,20). Time is particularly relevant when antibacterial activity depends partly on gradual dissolution or accumulation of cellular damage. Reporting concentration without sufficient information on expo-

sure duration therefore provides an incomplete description of the antibacterial challenge (1,5,9).

Increasing dose also raises an important question of selectivity. A concentration that produces strong bacterial inhibition may have limited practical value if it causes comparable damage to mammalian cells or non-target organisms. For biomedical applications in particular, antibacterial efficacy should therefore be considered in relation to the concentration range over which acceptable cytocompatibility is maintained (14,16,17,20).

Dissolution and Metal-Ion Release

For nanoparticles capable of dissolution, metal-ion release can substantially influence antibacterial performance. The nanoparticle in this case acts not only as a particulate material but also as a source of soluble metal species (21–25). The extent and rate of dissolution depend on the material composition, particle size, surface characteristics, and the chemistry of the surrounding medium (12,21,22,24,25). This relationship is particularly relevant to Ag-, Cu-, and Zn-containing nanoparticles, where released ions can interact with proteins, enzymes, membranes, and other cellular components (21–25). Smaller particles may dissolve differently from larger ones because of their greater specific surface area, illustrating how dissolution is linked to other physicochemical characteristics rather than functioning as an independent variable (12,22,25). Surface coatings may further restrict or modify contact between the particle and medium and consequently change ion availability (22,24,25).

4.1.3. Experimental Factors Dictating the Characteristics and Properties of MBNPs

Method of Synthesis

Chemical, physical, and biological or green synthesis methods can produce nanoparticles with different sizes, shapes, surface coatings, and aggregation behavior. The synthesis route can therefore influence antibacterial activity indirectly through the physicochemical characteristics of the final product. Residual reactants, reducing agents, stabilizers, and other compounds remaining after synthesis may also affect the measured biological response if purification is incomplete (8,26–28).

Biological synthesis using plant extracts, microorganisms, or biomolecules has received consid-

erable interest because biological components can act as reducing and stabilizing agents (8,26,29). Such approaches may reduce the need for some conventional chemical reagents and can produce nanoparticles carrying biologically derived surface molecules (26,29). At the same time, the composition of biological extracts is inherently complex and may vary with source and preparation conditions. Batch-to-batch reproducibility, identification of surface-bound compounds, purification, and precise control of particle properties therefore remain important considerations (8,20,26,29). It should be noted that biologically-based synthetic approaches most commonly yield spherical MBNPs (29).

Green synthetic approaches for MBNPs often result in richer surface functionalization than chemically produced MBNPs. The so-obtained “crown” on the surface often consists of bioactive compounds; therefore, in addition to being responsible for the reduction of metal ions to metal nanoparticles and their stabilization in colloidal dispersions, it may also contribute to the antimicrobial potency of the particles (8,26,29,30).

Comparing synthesis routes requires more than identifying a material as chemically, physically, or biologically produced. The properties of the resulting nanoparticles and the composition of their surfaces need to be considered. Otherwise, differences attributed to the synthesis method may actually reflect differences in particle size, morphology, surface-bound compounds, or residual components of the preparation (8,26,27,29,30).

Surrounding Environmental Conditions

Ionic strength, pH, temperature, organic matter, light exposure, and the composition of the surrounding medium can influence nanoparticle aggregation, dissolution, surface charge, and ROS production (9,25,31,32). Their importance varies by nanoparticle composition. Acidic conditions, for example, may alter the dissolution of some metal oxides, whereas light exposure is particularly relevant for photocatalytic materials such as TiO₂ and ZnO (9,25,31). Proteins and other organic molecules can adsorb onto nanoparticle surfaces, further modifying their biological identity and interactions with bacteria (32).

Once nanoparticles enter a complex biological medium, the surface initially characterized after synthesis may no longer be the surface presented to bacterial cells (16,32,33). Adsorption of proteins and other biomolecules can alter particle charge, colloidal stability, recognition, and interaction with cellular surfaces (7,16,32,33). The medium composition is therefore not merely a background experimental variable but can become part of the nanoparticle system itself (32,33).

Light exposure deserves separate consideration for photocatalytic materials (25,31,34,35). TiO₂ and, under appropriate conditions, ZnO can show altered ROS production following irradiation (25,31,34). Antibacterial results obtained under defined light conditions should therefore not be extrapolated directly to settings in which the intensity or wavelength of exposure differs substantially (31,34,35).

Bacterial Factors

Variation in antibacterial activity cannot be explained solely from the nanoparticle side of the interaction (10,15,36,37). The bacterial target also contributes substantially to the measured response. Differences in cell-envelope structure between Gram-positive and Gram-negative bacteria can influence nanoparticle adsorption, access to the cytoplasmic membrane, and susceptibility to surface-mediated damage (15,36,37). These structural differences are important, but they do not establish a universal rule whereby one bacterial group is consistently more susceptible to MBNPs than the other (10,36,37).

Variation can also occur between species and even between strains of the same species (14,36–39). Surface composition, stress-response systems, metal homeostasis, antioxidant defenses, efflux mechanisms, and other physiological characteristics may alter the response to nanoparticle exposure (14,15,38,39). The resistance phenotype of a strain should likewise be clearly reported, particularly when investigating multidrug-resistant clinical isolates (14,38,39).

The physiological state of the bacteria further affects susceptibility. Cells in different growth phases do not necessarily respond identically to antimicrobial stress, and bacteria within biofilms differ markedly from their planktonic counterparts (40–42). Biofilm-associated cells are embedded in an ex-

Table 1. Representative MBNP studies and key determinants of activity.

MBNP type	Shape	Size	Surface Charge	Surface modification	Target Bacteria	Key MIC Values or Antibacterial Outcomes	Reference
ZnO NPs	Granular/mixed shape	Mean size of about 48–52 nm (SEM); manufacturer-reported average size of 67 nm	Negative; numerical ζ -potential not reported	Not reported; commercial ZnO NPs	<i>S. aureus</i>	ZnO NP toxicity in Mueller–Hinton broth was mediated predominantly by dissolved Zn^{2+} ; medium composition strongly influenced the antibacterial response.	(9)
ZnO NPs	Not reported	22, 35, and 66 nm (TEM)	Not reported	None	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>S. aureus</i>	Antibacterial activity was size-dependent; with smaller ZnO NPs showing greater antibacterial activity than larger particles.	(11)
AgNPs	Spheres, platelets, cubes, and rods	Spheres glucose synthesis 40–70 nm (SEM); 45 nm (DCS); 108 nm (DLS); diameter: 145 nm, height: 48 nm (AFM); Spheres microwave synthesis and 120–180 nm (SEM); 123 nm (DCS); 128 (DLS); Platelets 20–60 nm; 30 nm (DCS); 48 nm (DLS); diameter: 80 nm, height: 16 nm (AFM); Cubes 140–180 nm; 167 nm (DCS); 211 nm (DLS); Rods 50–100 nm diameter, 1000–20000 nm length (SEM); 558 nm (DLS)	Spheres: –6 and –3 mV; Platelets: –7 mV; Cubes: –11 mV; Rods: –20 mV	PVP-stabilized	<i>S. aureus</i>	Antibacterial effects varied with particle size and shape despite comparable surface functionalization; activity was associated with Ag dissolution and specific surface area.	(12)
AgNPs	Spherical (AgNS) and truncated-octahedral (AgNOct)	AgNS average diameter–195 nm; AgNOct average diameter–194 nm (SEM)	Positive; numerical ζ -potential not reported	None	<i>E. coli</i> , <i>E. faecium</i>	Antibacterial activity differed according to particle shape; AgNOct showed stronger antibacterial effects than AgNS under the tested conditions.	(13)

AgNPs-chlorhexidine conjugate	Not reported	Unconjugated Ag NPs: 92.34 nm (DLS)	Ag NPs: -50.01 mV; after chlorhexidine conjugation: +44.59 mV	Green-tea-derived surface coating; chlorhexidine conjugation	<i>E. coli</i> , <i>S. aureus</i>	AgNP-chlorhexidine conjugate MIC: Ag 5.5 µg/mL + chlorhexidine 8.8 µg/mL against both bacteria. AgNPs alone had an MIC of 175 µg/mL against <i>E. coli</i> , while no MIC against <i>S. aureus</i> was established within the investigated range.	(20)
ZnO NPs	Not reported	< 100 nm	Not reported	Not reported; commercial ZnO NPs	<i>P. aeruginosa</i> , <i>Salmonella</i> spp., <i>K. pneumoniae</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>E. faecalis</i> , <i>S. saprophyticus</i> , <i>S. pyogenes</i>	MIC range: 15.625–125 µg/mL; MBC range: 62.5–250 µg/mL; inhibition zones 16–21 mm. Gram-positive isolates were generally more susceptible.	(28)
Pt, Ag, and Au NPs with HSA protein corona	Spherical	PtNPs: 10 ± 2 nm; PtNP-HSA: 30.5 ± 3 nm; AgNPs: 9 ± 3 nm; AgNP-HSA: 18.0 ± 3 nm; AuNPs: 10 ± 2 nm; AuNP-HSA: 21.6 ± 3 nm (DLS)	Not reported	HSA corona	<i>E. coli</i>	Formation of an HSA protein corona modified nanoparticle-bacterium interactions and altered antibacterial behavior.	(33)
Cu-doped / Ag-decorated TiO ₂ NPs	Irregularly shaped	TiO ₂ -A: 20.2 ± 5.7 nm (TEM), 19.81 ± 4.55 nm (SEM), 74.47 ± 23.9 nm (DLS); TiO ₂ -A:Cu-1: 21.9 ± 5.4 nm (TEM), 23.93 ± 6.2 nm (SEM), 33.55 ± 11.4 nm (DLS); TiO ₂ -A:Cu-2: 22.20 ± 5.52 nm (SEM), 57.41 ± 24.27 nm (DLS); TiO ₂ -A:Cu-3: 22.05 ± 4.85 nm (SEM), 357.9 ± 109.9 nm (DLS); TiO ₂ -B: 82.1 ± 18.2 nm (TEM), 89.4 ± 13.3 nm (SEM), 165.1 ± 96.76 nm (DLS); TiO ₂ -B:Ag-1: 140.4 ± 37.0 nm (TEM), 127.1 ± 44.2 nm (SEM), 167.3 ± 30.1 nm (DLS); TiO ₂ -B:Ag-2: 131.4 ± 24.0 nm (SEM), 154.3 ± 94.4 nm (DLS); TiO ₂ -B:Ag-3: 142.2 ± 31.7 nm (SEM), 142.8 ± 67.58 nm (DLS)	TiO ₂ -A: -13.2 ± 5.4 mV; TiO ₂ -A:Cu-1: -18.4 ± 4.24 mV; TiO ₂ -A:Cu-2: -22.8 ± 5.31 mV; TiO ₂ -A:Cu-3: -24.8 ± 6.56 mV; TiO ₂ -B: +25.3 ± 6.38 mV; TiO ₂ -B:Ag-1: -10.4 ± 5.73 mV; TiO ₂ -B:Ag-2: -54.9 ± 13.6 mV; TiO ₂ -B:Ag-3: -67.7 ± 11.1 mV	Cu doping or Ag decoration	<i>E. coli</i> , <i>S. aureus</i>	Modified TiO ₂ NPs showed enhanced antibacterial activity, particularly under visible-light irradiation, associated with enhanced photocatalytic/ROS-generating activity.	(35)

AgNPs	Spherical	2 nm	Not reported	Not reported; commercially obtained AgNPs	Selected Gram-positive and Gram-negative pathogens	AgNPs exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria, with differences in susceptibility among tested organisms.	(36)
C-phycocyanin-stabilized Ag NPs (Ag-Pc)	Hexagonal/spherical	Classic AgNPs : 175.25 nm (DLS); 15–25 nm (TEM); Ag-Pc: 215.6 nm (DLS); 26–43 nm (TEM)	Classic Ag NPs: –20.4 mV Ag-Pc: –18.1 mV	C-phycocyanin stabilization	MRSA; carbenem-resistant <i>P. aeruginosa</i>	Ag-Pc MIC: 7.4 µg/mL against both bacteria. MBC: 7.4 µg/mL against MRSA and 14.9 µg/mL against <i>P. aeruginosa</i> . Ag-Pc also showed greater antibiofilm activity than unmodified AgNPs.	(38)

Abbreviations: AFM—atomic force microscopy; AgNPs—silver nanoparticles; DCS—differential centrifugal sedimentation; DLS—dynamic light scattering; HSA—human serum albumin; MBC—minimum bactericidal concentration; MBNPs—metal-based nanoparticles; MIC—minimum inhibitory concentration; MRSA—methicillin-resistant *S. aureus*; PVP—polyvinylpyrrolidone; ROS—reactive oxygen species; SEM—scanning electron microscopy; TEM—transmission electron microscopy; TiO₂ NPs—titanium dioxide nanoparticles; ZnO NPs—zinc oxide nanoparticles.

tracellular matrix and display metabolic and physiological heterogeneity that can modulate nanoparticle access and antibacterial responses. Consequently, activity measured against planktonic cultures should not be used as a direct predictor of antibiofilm performance (10,38,40–42).

Representative experimental studies illustrating how major physicochemical, environmental, and biological determinants influence the antibacterial activity of MBNPs are summarized in Table 1. The reported antibacterial endpoints should be interpreted within the experimental conditions of each study rather than compared directly across studies, because nanoparticle characterization, bacterial strains, exposure conditions, and antibacterial assays differ considerably.

4.2. Interdependence of Factors

Although the factors discussed above are often investigated separately, their effects on antibacterial activity are closely interconnected (6,7,9,43,44). Particle size can influence dissolution and aggregation; surface coating can modify charge, colloidal stability, bacterial contact, and ion release; and the surrounding medium can alter these properties once the nanoparticle enters the test system (6,7,9,43). Concentration may likewise affect aggregation, while bacterial physiology and assay conditions determine how the resulting nanoparticle state is translated into a measurable antibacterial response. For this reason, attributing increased or decreased antibacterial activity to a single characteristic requires caution (6,7,43,44). A synthesis modification intended to change particle morphology may simultaneously alter size and surface chemistry (6,8). Similarly, two nanoparticle preparations with the same primary particle diameter may have very different effective sizes once dispersed in bacterial culture medium. Relationships between structure and antibacterial activity are therefore most informative when several physicochemical characteristics are measured together and under conditions relevant to the antibacterial experiment (7,9,43,44).

4.3. Implications for Characterization and Reporting

The variability described throughout this review highlights the need to characterize the material actually used in antibacterial testing rather than

relying only on nominal composition or manufacturer specifications (43–45). At minimum, studies should provide sufficient information on particle dimensions, morphology, surface properties, concentration, and coating or functionalization. Where relevant, aggregation state and dissolution behavior in the exposure medium should also be considered (6,7,43–45).

Microbiological conditions require comparable attention. Bacterial strain, inoculum, culture medium, exposure time, assay method, and endpoint should be clearly reported (43–45). For biofilm experiments, the stage and method of biofilm formation are also important (40–42,44). Such information does not eliminate biological variability, but it allows differences between studies to be interpreted more meaningfully (43–45).

A further consideration is the relationship between antibacterial activity and safety (14,20,46). For systems intended for biomedical use, reporting strong bacterial inhibition without evaluating the response of relevant mammalian cells provides only part of the information needed to assess potential usefulness (16,20,45,46). The concentration associated with antibacterial activity should therefore be considered in the context of the intended application and corresponding biological exposure (14,20,45,46).

5. DISCUSSION

The literature indicates that antibacterial activity is an emergent property of the complete nanoparticle–environment–bacterium system rather than a fixed characteristic of a particular metal or metal oxide. This helps explain why studies using nanoparticles of apparently similar composition can reach different conclusions. Nominal composition provides only the starting point; the form in which the material is presented to bacteria determines the interactions that can subsequently occur (5,7,9,32,43,44).

Among particle-related characteristics, size receives particularly extensive attention, but its influence highlights the difficulty of assigning activity to a single variable. A decrease in particle diameter increases specific surface area, yet the resulting biological effect may involve altered dissolution, surface reactivity, bacterial contact, or aggregation (6,7,11,12). Similar interdependence applies to morphology and surface functionalization (6,7,12,16). A coating intro-

duced to stabilize a nanoparticle may simultaneously change its charge, interaction with the bacterial envelope, and accessibility of the inorganic surface. Consequently, simple relationships such as “smaller particles are more antibacterial” are useful as general observations but insufficient as predictive rules (6,7,11,16).

The nanoparticle state during the antibacterial experiment is equally important. Characterization performed immediately after synthesis or in water does not necessarily describe the material present in a bacterial culture medium or biological fluid (7,9,32,33). Ionic strength can promote aggregation, proteins can adsorb to the surface, and dissolving nanoparticles may progressively generate soluble metal species (9,21,32,33). The antibacterial system therefore changes with time and with the composition of the surrounding medium (9,32,33,43). This distinction is particularly relevant when considering laboratory findings for biomedical or environmental applications (32,43–45).

Variation on the bacterial side further complicates interpretation (10,15,36,38). Differences between Gram-positive and Gram-negative envelopes can influence initial particle interactions, but Gram classification alone cannot predict bacterial susceptibility (15,36). Species, strain, physiological state, and biofilm growth can all modify the response (10,38,40,42). The use of a single laboratory strain may therefore be useful for controlled comparison but provides limited information about the range of activity expected against clinically or environmentally relevant bacterial populations (14,36,38,43).

Methodological differences are another important source of apparent disagreement. Diffusion-based, broth-based, time-kill, and biofilm assays do not measure identical outcomes, and nanoparticle-specific behavior can interact with the method itself (10,43,44). Limited diffusion through agar, sedimentation in liquid culture, optical interference, or gradual ion release can influence the result independently of bacterial susceptibility. Direct comparison of antibacterial values across studies is therefore most meaningful when the nanoparticle characterization and microbiological methodology are sufficiently similar (9,20,43–45).

These observations have practical implications for designing antibacterial nanomaterials (7,14,16,17). Optimization should not focus on maximizing a single characteristic such as small size, positive charge, or high concentration (7,16,17,20). Instead, the objective is to identify combinations of properties that provide sufficient antibacterial activity while retaining stability, selectivity, and an acceptable safety profile under the intended conditions of use. This is especially important in biomedical applications, where enhanced interaction with bacterial membranes may also increase interaction with mammalian cells (14,16,17,20,45).

Future work would benefit from experimental designs that deliberately examine interactions between variables rather than modifying one nominal parameter without characterizing the accompanying changes (7,43–45). Physicochemical measurements performed in the actual exposure medium, assessment of ion release where relevant, and transparent reporting of bacterial and assay conditions would improve interpretation (9,21,43–45). Studies incorporating clinically relevant strains, established biofilms, and parallel cytocompatibility assessment would further help determine whether physicochemical advantages observed in simplified systems remain useful under more realistic conditions (14,20,38,40,42,43).

The field therefore faces a shift from demonstrating antibacterial activity toward understanding and controlling it. Establishing reproducible relationships between material characteristics, exposure conditions, and biological response will be necessary to design MBNPs rationally rather than evaluate them largely on a formulation-by-formulation basis (7,43–45).

6. CONCLUSION

The antibacterial activity of MBNPs is determined by more than their chemical composition. Particle size and morphology, surface properties, aggregation, concentration, dissolution, and synthesis all contribute to the form and reactivity of the material presented to bacterial cells. These characteristics are further modified by the surrounding medium and interact with bacterial and experimental variables, making antibacterial performance difficult to predict from any single parameter.

A better understanding of these relationships is needed to explain differences between published studies and to guide the rational design of antibacterial nanomaterials. Future investigations should combine thorough physicochemical characterization with clearly defined microbiological conditions and, where possible, characterize nanoparticle behavior in the medium in which antibacterial activity is actually measured. For biomedical applications, antibacterial efficacy should also be evaluated together with cytocompatibility rather than as an isolated endpoint. Greater consistency in characterization and reporting would make comparisons between studies more meaningful and help identify which combinations of nanoparticle properties are genuinely advantageous for antibacterial use.

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