Emerging nanotechnology-based antimicrobials against antimicrobial-resistant gram-negative bacteria: Mechanisms, therapeutic advances, and translational challenges

Supplementary File

Table S1. Data extraction table

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
1.	Moosazadeh Moghaddam *et al.*[1]	Iran	Silver nanoparticles (AgNP)	*P. aeruginosa*, *A. baumannii*	Randomized controlled trial	A promising alternative to antibiotics for multidrug-resistant (MDR) infections	Destroys germs while preserving healthy tissue	A targeted, effective option for drug-resistant infections with low resistance risk and minimal microbiome disruption	Small sample size and no long-term follow-up to evaluate lasting effectiveness and safety
2	Chen *et al.*[2]	China	Titanium dioxide (TiO_2)/AgNP	*E. coli*	Experimental laboratory-based study	Enhanced membranes effectively removed tetracycline-resistant genes (TRGs) and *E. coli* from wastewater	Silver ions and reactive oxygen species (ROS) destroyed bacteria by damaging their membranes and metabolism	Boost antibacterial action, TRG rejection, and biofouling resistance	Using wastewater from one source limited the study's generalizability
3	Huang *et al.*[3]	China	Selenium NPs	*E. coli*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*	Experimental laboratory-based study	Enhanced effectiveness against superbugs by damaging cell membranes and causing bacterial cell death	Damaged bacterial membranes led to leakage and cell death	Offers stronger antibacterial activity against superbugs than conventional methods, with lower toxicity and reduced risk of resistance	Absence of *in vivo* testing to confirm safety and efficacy
4	Nithiyavathi *et al.*[4]	India	Copper oxide (CuO) NPs	*K. pneumoniae*, *P. mirabilis*, *Salmonella* Typhimurium, *E. coli*	Experimental laboratory-based study	Showed strong antibacterial activity	Disrupted bacterial cell membranes, causing leakage and cell death	Provided a cheaper, greener, and more effective alternative to traditional antimicrobial treatments	Lacked data on durability, toxicity, and *in vivo* effects
5	Manzoor *et al.*[5]	India	Platinum (Pt) NPs	*P. aeruginosa*, *K. pneumoniae*, *E. coli*	Experimental study (*in vitro*)	Strong antibacterial activity	Caused DNA damage and oxidative stress, disrupting bacterial membranes	Eliminates harmful chemicals, making it eco-friendly and cost-effective	Long-term toxicity and *in vivo* effects of PtNPs were not evaluated
6	Huang *et al.*[6]	Australia	Selenium NP coated with ε-poly-L-lysine	*E. coli and* *K. pneumoniae*	Experimental *in vitro* study	Blocked resistance, killed bacteria effectively, and were minimally toxic to human cells	Damaged membranes generate ROS and disrupt cell functions	Safer, sustainable alternative to antibiotics with broad action, low toxicity, and low resistance risk	Lacked *in vivo* testing and long-term safety, toxicity, and scalability assessments
7	Chaurasia *et al.*[7]	South Korea	Amino-functionalized silica-coated magnetite magnetic core-shell NPs	*E. coli*	Experimental *in vitro* study	Destroy bacteria's membranes without causing resistance	Trapped and killed bacteria via membrane disruption under radiofrequency	Eliminates drug-resistant bacteria without causing resistance or biofilms	Lack of *in vivo* testing and scalability challenges
8	Goel *et al.*[8]	India	AgNPs from *Streptomyces* spp. EMB24	*P. aeruginosa* and tetracycline-resistant *Neisseria gonorrhoeae*	Experimental laboratory-based study	Blocked biofilms and drug-resistant bacteria, showing medical potential	Break cell walls, release ions, generate ROS, and block biofilms	Target resistant bacteria and biofilms without causing resistance	*In vitro* results may not reflect real-world clinical effectiveness or safety

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Table S1. (*Continued*)

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9	Arya *et al.*[9]	India	Vanillin-capped gold (Au) NPs	*P. aeruginosa* (extensively drug-resistant (XDR) clinical isolates)	Experimental *in vitro* study	Boosted antibiotic effectiveness against XDR *P. aeruginosa*, lowering minimum inhibitory concentrations (MICs) by up to 14-fold.	Blocked efflux pumps boost antibiotic uptake	Served as antibiotic adjuvants, enhancing efficacy without directly killing bacteria	*In vitro* only; *in vivo* validation is needed for clinical use
10	Ashajyothi *et al.*[10]	India	Biogenically synthesized copper (Cu) NPs and zinc oxide (ZnO) NPs were used	*E. coli* and *P. aeruginosa*	*In vitro* experimental laboratory study	Showed strong antibacterial and antibiofilm effects, especially with antibiotics	Boosted antibiotic penetration, generated ROS, and disrupted bacterial membranes	More effective against resistant bacteria and needed lower doses than traditional antibiotics	Limited to laboratory tests, lacking *in vivo* or clinical validation
11.	Ghasemi *et al.*[11]	Iran.	AgNPs synthesized using Rubus discolor leaf extract.	MDR *E. coli* and *P. aeruginosa*	Experimental study	Exhibited potent antibacterial and anticancer properties, along with excellent stability and biocompatibility	ROS, cell membrane disruption, and interference with cell signaling	Cost-effective, eco-friendly, and avoids harmful chemicals used in the conventional method	*In vivo* or clinical validation is still needed
12	Sanyasi *et al.*[12]	India	Carboxymethyl tamarind polysaccharide-capped AgNPs	*E. coli*, *Salmonella* Typhimurium, MDR clinical strains	Experimental laboratory-based study	Inhibited bacterial growth and biofilms with minimal toxicity to mammalian cells	Disrupted bacterial cell division by targeting FtsZ and FtsA proteins	Targeted MDR bacteria and biofilms with lower toxicity than conventional antibiotics	Limited to *in vitro* results; *in vivo* or clinical validation is still needed
13.	Morellá-Aucejo *et al.*[13]	Spain	Mesoporous silica (MSN) NPs functionalized with ε-poly-l-lysine.	*E. coli*	*In vitro* experimental study	Increased antibacterial effectiveness at incredibly low dosages	Disrupted microbial cells and raised local concentration	Boosted antibacterial effectiveness up to ×60 th controlled, targeted cinnamaldehyde release	Limited to *in vitro* findings, lacking *in vivo* and long-term environmental safety data
14.	Cai *et al.*[14]	China	Curcumin-stabilized AgNPs	*E. coli*	*In vitro* experimental study	Showed sustained antimicrobial effects, disrupting biofilms and reversing drug resistance	Membrane rupture, ROS generation, and metabolic suppression	Broad-spectrum action, low toxicity, and the ability to reverse resistance genes, such as *sdrM*	Lacks long-term biocompatibility and *in vivo* evaluations needed for clinical use
15	Sathishkumar *et al.*[15]	India	AgNP	*E. coli*	Experimental study	The biosynthesized AgNPs showed strong antioxidant, antibacterial, and anticancer activities, proving especially effective against drug-resistant infections	Ruptured microbial membranes, generated ROS, and induced cancer cell apoptosis	Cost-effective, eco-friendly, and avoids toxic chemicals used in traditional NP production	Lack of long-term toxicity studies and *in vivo* testing
16	Zhu *et al.*[16]	China	AgNPs decorated on MSN-coated single-walled carbon nanotubes	*E. coli*	*In vitro* and *in vivo* experimental study	Improved antibacterial activity, efficient wound healing, and good biocompatibility	Disrupted bacterial membranes and rapidly released silver ions	Offer improved dispersibility, controlled Ag release, and synergistic effects	Complex synthesis may hinder clinical use and large-scale production

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Table S1. (*Continued*)

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17	Yao *et al.*[17]	China	AuNPs modified with derivatives of mercaptobenzimidazole.	*E. coli*	Experimental laboratory study	Broad-spectrum antibacterial activity	Lower adenosine triphosphate (ATP) levels, rupture of cell membranes, and collapse of the membrane potential	Offer a non-antibiotic alternative capable of overcoming bacterial drug resistance	Electroneutral 5-methyl-2-mercaptobenzimidazole-AuNPs and 5-ethoxy-2-mercaptobenzimidazol-AuNPs were less effective than methoxy-2-mercaptobenzimidazole-AuNPs
18	Alzoubi *et al.*[18]	Jordan	AgNPs, magnetite (Fe3O4) NPs, and Fe_3O_4/Ag core-shell NPs	*Salmonella* Typhimurium, *P. aeruginosa*	Experimental study	Fe_3O_4/Ag core-shell NPs were the most effective, though all showed antibacterial activity	Induce oxidative stress and disrupt bacterial cell membranes	Strong effectiveness against drug-resistant bacteria	Need to assess NP cytotoxicity and long-term safety
19	Hashemi *et al.*[19]	Iran and Iraq	Biosynthesized AgNPs produced using *Menthapulegium* and *Crocus caspius* plant extracts.	MDR strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*	Experimental study.	Strong antibacterial and anticancer effects, with *M. pulegium*–AgNPs having the lowest MIC	Ruptured microbial cell membranes and caused cytotoxicity in cancer cells	An eco-friendly, cost-effective, and non-toxic alternative to traditional chemical processes	Lack of full toxicity analysis and *in vivo* testing limits clinical translation
20	Pramanik *et al.*[20]	United States	The nanocomposite consists of polydopamine NPs, graphene oxide, and ε-poly-l-lysine	*K. pneumoniae*	Experimental study	Completely eliminated XDR bacteria in the contaminated water samples	Used ε-poly-l-lysine to kill superbugs and surface groups to adsorb metal ions	Combined disinfection and purification in one system	The study did not assess toxicity, scalability, or long-term stability of the composite
21	Xue *et al.*[21]	China	AgNPs, Fe_3O_4/Ag coated NPs, polydopamine–graphene oxide–ε-poly-l-lysine, and mixed-charge hyperbranched polymer NPs	*K. pneumoniae*, *E. coli*, and *P. aeruginosa*	Experimental (*in vitro* and *in vivo* studies)	Optimized mixed-charge hyperbranched polymer NPs exhibited high selectivity, inhibited biofilm formation, delayed the emergence of resistance, and was safe for mammalian cells	Selective membrane disruption based on bacterial versus mammalian membrane potential differences	Highly selective, biocompatible, avoids metal ion/ROS toxicity, and usable as an infection-preventing coating	Further research is needed to clarify the mixed-charge system's structure–function relationship
22	Jain *et al.*[22]	India	AgNPs synthesized using Azadirachtaindic, a (neem) leaf extract.	*B. licheniformis*, *K. pneumoniae*, and *E. coli*	Experimental study	Strong antibacterial activity, excellent ultraviolet protection, and high wash durability	Damaged bacterial membranes and DNA by releasing Ag ions	Eco-friendly, non-toxic, and washable alternative to conventional antimicrobial treatments	Some bacteria, such as *E. coli*, were moderately affected, and the effect declined after multiple washes
23	Najafi *et al.*[23]	Iran	Amine-functionalized MSN NPs	MDR *A. baumannii*	Experimental study	Had much higher antibacterial activity in comparison to free medicines	Enhanced antibiotic effectiveness by providing a high local concentration via controlled release	Reduced toxicity and dosage while improving drug stability, bioavailability, and efficacy	Adverse effects *in vivo* were not evaluated because the study was conducted *in vitro*

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24	Xie[24]	China.	AgNP	MDR bacteria, including *E. coli*	Experimental laboratory-based study	Decreased the production of biofilms and inhibited MDR microorganisms	Low toxicity and delayed resistance by disrupting growth and biofilms	Lower resistance risk, low toxicity, and strong MDR and biofilm inhibition	No clinical or *in vivo* validation for long-term safety and effectiveness
25	Yaseen *et al.*[25]	India,	Palladium NPs from *Cannabis sativa* leaf extract.	MDR bacteria (*E. coli*, *K. pneumoniae*)	Experimental *in vitro* study	Significant antibacterial activity and cytotoxicity against A549 cells	Disrupted bacteria and cancer cells by targeting macromolecules and inducing oxidative stress	Economical, environmentally benign, and free of hazardous chemicals compared to conventional approaches	*In vitro* only, lacking *in vivo* validation and full toxicity profiling
26	Akbar *et al.*[26]	Pakistan	Cinnamic acid-coated magnetic iron oxide and MSN NPs	MDR *E. coli* and *P. aeruginosa*	Experimental *in vitro* study	Stronger antibacterial action, eliminating resistant germs at lower dosages	Enhanced drug delivery and absorption without raising human cell toxicity	Reduced required doses and may prevent resistance by boosting antibiotic efficacy	Lacks clinical and *in vivo* studies to confirm *in vitro* results and assess long-term safety and efficacy
27	Hodhod *et al.*[27]	Saudi Arabia.	AgNPs from the marine fungus *Amarenographium solium*	MDR strains of *E. coli* and *P. aeruginosa*	*In vitro* experimental study	Strong antibacterial activity with MIC as low as 9.375 µg/mL	Oxidative stress and membrane disruption	Produces high-yield, bioactive NPs safely, scalable, economical, and eco-friendly.	Limited to *in vitro*; *in vivo* validation and safety checks are needed for clinical use
28	Afolayan *et al.*[28]	Nigeria	AgNP from *Bacillus thuringiensis*	*E. coli* and *K. pneumoniae*	Experimental laboratory-based study	Showed strong antibacterial activity against most drug-resistant microbes except one *E. coli* strain	Damage bacterial membranes and obstruct essential cellular functions	A safe and efficient substitute for conventional antibiotics	Further research is needed on the environmental effects on AgNP properties
29	Mendez-Pfeiffer *et al.*[29]	Mexico	AgNP from methanolic extracts of Sonoran Desert propolis	MDR clinical isolates of *E. coli*	Experimental study	Demonstrated low cytotoxicity and potent antibacterial and antibiofilm action	Breaks bacterial membranes and suppresses biofilm formation	Eco-friendly antibacterials, avoiding harmful chemicals and reducing toxicity	Long-term toxicity evaluations and *in vivo* assessments were not included in the study, which was restricted to *in vitro* experiments
30	Thabet *et al.*[30]	Egypt	CuONPs	*P. aeruginosa*, *K. pneumonia*, *E. coli*, *A. baumannii*, *E. aerogenes*, *P. mirabilis*, *Burkholderia cepacia*	Cross-sectional study	Concentration-dependent biofilm inhibition up to 97.1%, effective across all tested species	ROS generation, DNA degradation, disrupted bacterial metabolism, and transport	Cost-effective non-antibiotic options for treating MDR infections	No clinical trial data, *in vitro* only, and some data was based on a single isolate
31	Sabzevar *et al.*[31]	Iran	Silver chloride (AgCl) NP	*E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853)	Experimental laboratory-based study	Showed strong antibacterial activity, low MICs, cytotoxicity against HepG-2 and MCF-7, and superior antioxidant activity	Ag^+ damaged DNA/proteins, generated ROS; enzymes and amino acids aided reduction and stabilization	Eco-friendly, cost-effective, and biocompatible with natural stabilizers showed antibacterial and anticancer effects	Lacks *in vivo* validation, shows strain-specific sensitivity, and needs more study on AgCl formation and cytotoxicity

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Table S1. (*Continued*)

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32	Yu *et al.*[32]	China	University of Oslo metal–organic framework (UiO-66), modified to MXF@ UiO-UBI-PEGTK, incorporating moxifloxacin (MXF), the targeting peptide UBI29-41, and the PEG derivative Pro–Glu–Gly–Thr–Lys (PEGTK) for coating and stability	*P. aeruginosa*	Experimental study	Showed high drug loading, hydrogen peroxide (H_2O_2)-responsive release, strong biofilm inhibition, better targeting, retinal cell safety, and reduced infection in rabbits	MXF blocked DNA replication, UiO-66 provided controlled delivery, UBI29-41 targeted membranes, and PEGTK enabled oxidative-responsive release for deep biofilm penetration and bacterial eradication	Provided targeted delivery, controlled release, deep biofilm penetration, better stability, biocompatibility, and synergistic effects	Lacked long-term safety, pharmacokinetics, and immunogenicity data; the data were preclinical and had scalability issues from complex synthesis
33	Gharieb *et al.*[33]	Egypt	AgNP	*Pseudomonas* spp., *Klebsiella* spp., *E. coli*, *Proteus* spp.	Experimental laboratory-based study	Strong antibacterial effect, outperforming Ag nitrate and ginger extract.	Penetrated membranes, triggered ROS, disrupted DNA/ enzymes, and caused scanning electron microscopy (SEM)/ transmission electron microscopy (TEM)-visible damage.	Eco-friendly, cost-effective synthesis, and broad MDR activity	Lacked *in vivo* tests, had variable strain efficacy, no long-term safety data, and limited insight into molecular mechanisms
34	Gideon and Aliyu Dikko[34]	Nigeria	Aluminium (Al) NPs	*E. coli*	Experimental study	AlNPs using rice straw extract showed dose-dependent activity, with *E. coli* zones from 2.0 mm (25 μg) to 14.8 mm (100 μg)	ROS damaged membranes; electrostatic interactions and functional groups aided NP formation and stabilization	Eco-friendly synthesis, avoided harmful chemicals, was effective against drug-resistant bacteria, and offered a cost-effective antimicrobial solution	The study had agglomeration, uneven distribution, condition-dependent results, and no *in vivo* safety or efficacy data
35	Abou Elez *et al.*[35]	Egypt	AgNP	*S. enteritidis*, *Salmonella* Typhimurium	Experimental laboratory study	Strong activity with large inhibition zones, low MIC/minimum bactericidal concentration (MBC), gene suppression, and full inhibition in 48 h	Penetrated cell walls (27 nm), disrupted membranes, suppressed virulence/ resistance genes, and generated ROS	Eco-friendly, broad MDR activity, low resistance risk, stable particles, and potential as antibiotic alternatives in poultry farming	Lack *in vivo* testing, long-term safety data, and show variable effectiveness by dose, strain, and environment
36	Mala and Ruby Celsia[36]	India	AgNP	*E. coli*	Experimental laboratory study	Showed strong antimicrobial activity (32 mm zone versus *E. coli*) and effectiveness against biofilm-forming, resistant superbugs	Disrupted membranes, bound protein thiols, interfered with DNA replication, and generated ROS damaging biomolecules and enzymes	Effective against MDR biofilm pathogens, with multi-action mechanism, thermal stability, crystalline structure, and burn dressing potential	*In vitro* only, lacked *in vivo* or clinical trials, and tested just two bacterial species from one sample

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37	Novickij *et al.*[37]	Lithuania	Nisin-loaded iron oxide NPs were capped with citric acid, ascorbic acid, and gallic acid	*E. coli*	Experimental laboratory study	Pulsed electric fields (PEF) and pulsed electromagnetic fields (PEMF) enhanced nisin-NP effect and cut *E. coli* by 3-log colony-forming units; PEMF alone had minimal effect but synergized to overcome resistance.	Nisin bound lipid II to form pores; PEF electroporated membranes for uptake; PEMF induced fields and hyperthermia to disrupt walls and move NPs	Overcame nisin resistance, provided non-thermal contactless treatment, reduced dose/time via synergy, and improved nisin stability and delivery with NP encapsulation	PEMF or NPs alone were less effective without PEF; synergy reduced in stationary phase or with *B. subtilis*, and PEMF–cell interaction needs further study
38	Achamo *et al.*[38]	Ethiopia	CuONPs	*P. aeruginosa*, *E. coli*	Experimental study	Strong antimicrobial activity and DNA binding, suggesting potential for gene interaction applications	Disrupted membranes, generated ROS, and penetrated cells to damage key biomolecules	Eco-friendly, biocompatible, small NPs with high surface area, strong MDR activity, and multifunctional therapeutic potential	Needs *in vivo* validation, as activity was sometimes lower than standard drugs such as fluconazole
39	Alamri *et al.*[39]	United States of America (USA)	Gallium (Ga) hydrogen phosphate NPs	*P. aeruginosa*	Experimental study	Potent antibacterial activity (MIC 8 μg/mL) without resistance after 30 passages, unlike gallium nitrate, $Ga(NO_3)_3$, and ciprofloxacin	Ga^{3+} mimicked iron (III) (Fe^{3+}), disrupted Fe metabolism, and controlled release boosted efficacy while avoiding $Ga(NO_3)_3$ hydrolysis	The formulation provided sustained Ga^{3+} release, better stability, biocompatibility, resistance prevention, and easy aqueous synthesis	*In vitro* only, no *in vivo* or cytotoxicity data included
40	Asvar *et al.*[40]	Iran	Chitosan-based nanocomposites incorporating Ag, CuO, ZnO, their binary combination and ternary combination	*P. aeruginosa*, *E. coli*, *and A. baumannii*	Experimental study	Strong antimicrobial activity, fibroblast-safe, and improved wound healing and bacterial reduction in rats	Chitosan-based nanofibrous mat combined with Ag, CuO, and ZnO NPs—damaging DNA/proteins, disrupting metabolism and membranes, enhancing oxidative and intracellular damage	Broad MDR activity, promoted wound healing, was fibroblast-safe, and reduced resistance risk by avoiding antibiotics	Lacked detailed synergy mechanisms, long-term multi-metal NP effects, and scalability or cost analysis
41	Ghasemi and Jalal[41]	Iran	ZnONPs	*A. baumannii*	Experimental study	Strong activity, combined with ciprofloxacin or ceftazidime, improved efficacy and uptake, and caused *A. baumannii* morphological changes (as observed by SEM)	Increased permeability, inhibited efflux pumps, damaged membranes, altered morphology, but no DNA fragmentation targeting membranes and transport, not genotoxicity	Boosted antibiotic efficacy against MDR pathogens by bypassing resistance; low doses to cut toxicity, and simple combination therapy without new drugs	*In vitro*, needs *in vivo* validation, assessment of long-term toxicity, and immune responses

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Table S1. (*Continued*)

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42	Žalnėravičius *et al.*[42]	Lithuania	Ultra-small methionine-capped AuNPs (~1.8 nm) and Au-functionalized Fe_3O_4NPs	*A. baumannii* (ATCC BAA-747), *S. enterica* (GTC-BTL, B-25)	Experimental study	Strong, concentration-dependent antimicrobial activity, while methionine and Fe_3O_4 alone showed no effect	Surface Au^+ ions disrupt membranes, metabolism, and proteins; inert Au^0 stays biocompatible; methionine acts as a safe stabilizer	Strong activity against resistant bacteria, excellent biocompatibility, good dispersibility, and low-dose potential	Lacked *in vivo* validation, had lower-dose efficacy limits, limited mechanistic insight, and no long-term safety or environmental assessment
43	Ilangovan *et al.*[43]	India	AgNP infused with *Azadirachta indica* (neem) extract	*P. gingivalis*, *T. denticola*, *T. forsythia*	Experimental/ comparative study	Strong antibacterial activity, highest against *T. forsythia* (19.3 mm zone, MIC 3.12 µg/mL, relative inhibitory zone 180), followed by *T. denticola* and *P. gingivalis*	Membrane and biofilm disruption, thiol-protein interaction, ROS generation, and gedunin activity	Superior to chlorhexidine, eco-friendly, effective against invasive pathogens, and suitable for drug delivery with better solubility, stability, and distribution	Limited to *in vitro* MIC/MBC tests with no clinical validation or cytotoxicity data, requiring further research and trials
44	Oliveira *et al.*[44]	Brazil	Tea tree oil (TTO) and chitosan NPs	*Aggregatibacter actinomycetemcomitans*	Experimental *in vitro* study	Synergistic activity, significantly reducing MIC values, inhibiting biofilms as effectively as azithromycin, with TTO being microbiostatic and chitosan microbicidal	TTO disrupts membranes and metabolism; chitosan alters permeability, binds DNA, and inhibits efflux pumps to overcome resistance	Natural, biocompatible, effective against resistant oral pathogens, matches azithromycin in biofilm inhibition with lower toxicity and > 70% fibroblast viability at ≤ 0.5 mg/mL	*In vitro* only, no *in vivo*/ clinical validation; TTO cytotoxic > 0.5 mg/mL, limited alone, needs more research on long-term safety and formulation efficacy
45	Amini *et al.*[45]	Pakistan	ZnONPs	Avian pathogenic *E. coli* (APEC)	*In vitro* experimental study	All APEC isolates were MDR, resistant to nalidixic acid, chloramphenicol, and streptomycin, while ZnONPs (MIC 32–256 mg/mL) inhibited them and suppressed *cas3*, thereby lowering the risk of resistance	Act through the release of zinc (Zn^{2+}) ions targeted to disrupt bacterial membrane integrity	Effective against MDR strains, minimally affect clustered regularly interspaced short palindromic repeats resistance genes, and act as both an antibacterial and bioavailable zinc source for poultry	*In vitro* only, did not assess virulence in commensal *E. coli*, and lacked long-term safety, efficacy, and toxicity data in live poultry
46	Ahmed *et al.*[46]	Iraq	AgNP, ZnNP, and their combination as Ag–Zn composite NPs	*K. variicola*, *Pantoea ananatis*	*In vitro* and *in vivo* experimental study	Exhibited strong antibacterial activity and significantly accelerated wound healing (reducing recovery time by over 50%) without observed toxicity at doses up to 5,000 mg/kg.	Bacterial membrane disruption, penetration into cells, metal ion release, and ROS generation lead to protein denaturation, DNA damage, and bacterial death, while also promoting collagen synthesis and skin epithelialization	Fights MDR pathogens, shortens healing time, is non-toxic, cost-effective, eco-friendly, and provides dual antimicrobial action	Lacked long-term safety, resistance data, and human clinical trials due to small *in vivo* sample size

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47	Kamer *et al.*[47]	Egypt	AgNP	*P. aeruginosa*	*In vitro* experimental study	Strong antimicrobial activity (MIC 2.65–21.25 μg/mL), synergized with gentamicin, ceftazidime, and ciprofloxacin, and inhibited biofilm formation	Disrupted biofilms, reduced virulence factors, altered quorum-sensing genes, and likely induced ROS and membrane damage	Enhanced antibiotic efficacy against MDR/XDR strains, with low cytotoxicity and potential for lower doses to cut toxicity and resistance	Lacked *in vivo* testing, used limited strains, and needs long-term safety and resistance studies
48	Yu *et al.*[48]	China	Colistin-loaded polymeric NPs	*P. aeruginosa*	*In vitro* and *in vivo* experimental study	Reduced biofilm, inhibited growth, improved wound healing, with low cytotoxicity and better drug release	Size and surface properties improved uptake, released colistin, boosted local concentration, penetrated biofilms, and disrupted membranes, causing cell death	Enhanced antibiofilm action, lowered cytotoxicity, improved wound healing, and enabled controlled drug release for better penetration	Lacked long-term toxicity, pharmacokinetics, clinical trials, and needs further study on mechanism and scalability
49	Tegegne *et al.*[49]	Ethiopia	Iron oxide (IO) NPs	Ciprofloxacin-resistant *E. coli*	*In vitro* experimental study	IONPs loaded with ciprofloxacin showed enhanced delivery, protected ciprofloxacin from resistance, and increased permeability	ROS, wall disruption, photocatalysis, DNA damage, and Fenton reactions	Reversed ciprofloxacin resistance in *E. coli* via sustained, targeted release, with eco-friendly, low-cost banana peel synthesis and reduced dosing through dual-phase release	Lacked *in vivo* studies, structural confirmation (TEM/Raman), and was tested on one bacterial species
50	Amedu *et al.*[50]	Nigeria	Green-synthesized AgNP	*E. coli*, *Salmonella* Typhi	*In vitro* experimental study	Potent antibacterial activity with a higher zone of inhibition than leaf extract and Ag nitrate solution alone	Small size and large surface area allowed penetration and membrane damage, disrupting permeability and energy systems	Eco-friendly, non-toxic, cost-effective, with higher antibacterial activity than extract alone, effective against Gram-negatives like ciprofloxacin	Toxicity unassessed; further study needed on antifungal, antioxidant activity, and *in silico* absorption, distribution, metabolism, excretion, and toxicity
51	Asif *et al.*[51]	Saudi Arabia	ZnONPs and Al-doped ZnO nanocomposites	*E. coli*, *P. multocida*	*In vitro* experimental study	Strong antimicrobial and anticancer activity against HepG-2, MCF-7, and SKOV3 cell lines, with half-maximal inhibitory concentration of 20.47 μg/mL	Damages membranes, DNA, and enzymes, blocking biofilms, inducing oxidative stress, cell cycle arrest, and inhibiting angiogenesis	Broad Gram-negative activity, low toxicity to normal cells, effectiveness at low doses, and improved cellular uptake	Lacked *in vivo* data and comprehensive toxicity profiling, requiring further research on long-term safety and mechanisms

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52	Vanti *et al.*[52]	India	Chitosan–AgNPs	*E. coli*	*In vitro* experimental study	Showed strong antibacterial, antioxidant, and wound healing effects, with 80% HEK-293 cell viability and 97% wound closure at 0.55 μg/mL	Disrupts bacterial membranes, inhibits biofilm formation, reduces ROS, and supports wound healing and antioxidant effects	Provided antibacterial, antioxidant, and healing benefits with low toxicity, high biocompatibility, and effective at lower doses than standard antibiotics	Lacked *in vivo* studies, with unresolved scalability and formulation challenges, requiring further research on safety and mechanisms
53	Mohamed[53]	Japan	CuONP	*K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *E. coli*, *A. baumannii*	*In vitro* experimental study	Showed superior antimicrobial and antibiofilm effects over vancomycin and amphotericin B, with good human cell compatibility.	Disrupted bacterial membranes, caused leakage, impaired DNA/RNA synthesis, and induced ROS damage	Broad-spectrum activity with reduced MIC/ minimum biofilm inhibitory concentration and improved safety for human cells	Effective *in vitro*, but requires *in vivo* and clinical validation due to safety and biocompatibility concerns
54	Pourrafsanjani *et al.*[54]	Iran	AgNPs stabilized on iron oxide NPs coated with carboxymethyl cellulose (CMC)	*E. coli*, *K. pneumoniae*, *Citrobacter* spp., *P. aeruginosa*	*In vitro* experimental study	Showed strong antibacterial and anti-biofilm effects, outperformed ciprofloxacin, and had low cytotoxicity.	Disrupted membranes, blocked enzymes, and induced cell death. CMC layer prevented adhesion, boosted biocompatibility, and enabled magnetic targeting	Effectively targeted resistant pathogens, had lower MIC than ciprofloxacin, and offered biocompatibility and biodegradability	*In vitro* results showed increased cytotoxicity at concentrations above 25 μg/mL AgNP; no *in vivo* data are available.
55	Rilievo *et al.*[55]	Czech	Surface active maghemite NPs (SAMNs)	*A. veronii*, *Phingomonas*, *Stenotrophomonas*, *Ralstonia*, *Cupriavidus*, *Methylobacterium*, *Williamsia*	*In vitro* experimental study	Achieved 100% bacterial removal in wastewater, with no toxicity, stable binding, reusability, and broad-spectrum action, including resistant strains	Captured bacterial lipopolysaccharides and glycosphingolipids via high-affinity binding to SAMN iron (III) sites, enabling magnetic removal without killing	Non-toxic, non-biocidal, binds broadly to bacteria, eco-friendly, low-cost, effective at low doses, and easily regenerable without surface modification	It removed bacteria but did not kill them; it may aggregate over time or at high doses, and it requires magnetic separation equipment
56	Malaikozhundan *et al.*[56]	India	ZnONPs	*E. coli* and *K. pneumonia*	*In vitro* study	Exhibited stronger activity against Gram-positive bacteria and boosted ciprofloxacin efficacy when loaded onto ZnO NPs	Disrupted membranes, generated ROS, and inhibited biofilm formation	Showed both antibacterial and antioxidant effects, boosting activity against drug-resistant bacteria	Results require *in vivo* validation; ZnONP toxicity and long-term effects remain unassessed
57	Hussein *et al.*[57]	Iraq	AgNP	MDR bacteria	Experimental study	Effective against MDR pathogens; higher doses caused dose-dependent liver and kidney toxicity in rats	Disrupted membranes, blocked protein synthesis, and triggered ROS, causing cell death	Effective against MDR pathogens, broadly antimicrobial, more stable, and less toxic than chemically made NPs	Bacterial strains were unspecified; dose optimization is needed for safe, effective use

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
58	Abdelrahman *et al.*[58]	Egypt	ZnONPs	*E. coli*, *P. aeruginosa*	Experimental *in vitro* antimicrobial study	Inhibited all tested pathogens with moderate to comparable efficacy to standard treatments	Disrupted microbial cell walls and showed possible synergy with crude extract bioactives	Showed broad-spectrum activity against bacteria and fungi, potentially curbing antibiotic resistance via multiple mechanisms	Lacked synergy analysis, *in vivo* validation, cytotoxicity data, and tested only at 1 mg/mL
59	Lokhande *et al.*[59]	India	*In situ* nanosuspension of PPEF.3HCl-bisbenzimidazole-based antibacterial agent	*K. moniae*, *A. aumannii*, *P. aeruginosa*, and *Enterobacter* species	Experimental study	The nanosuspension, featuring bi/trimodal particles and high precipitation, boosted macrophage uptake 1.6×, remained serum-stable, and enhanced antibacterial effects	Targets bacterial topoisomerase IA, disrupting DNA relaxation and replication	Nanosizing enhanced pharmacokinetics, boosted intracellular uptake, and improved efficacy against multidrug-resistant strains	Further studies are needed on long-term toxicity and pharmacodynamics. Limited ~5-h stability may affect shelf-life, requiring on-site preparation
60	Chand and Kushawaha[60]	India	Silibinin-loaded chitosan-capped silver NPs	*P. aeruginosa* and *Klebsiella pneumoniae*	*In vitro* study	Displayed strong antimicrobial and anti-biofilm effects against *P. aeruginosa* and *K. pneumoniae*, while reducing inflammation in lipopolysaccharide-stimulated THP1 cells.	AgNP exhibited antimicrobial activity by disrupting membranes, inhibiting biofilm formation, reducing inflammation, and preventing matrix formation	Effective against resistant strains with dual antibacterial and anti-inflammatory effects, biofilm inhibition, and reduced toxicity via natural capping	Despite *in vitro* success, *in vivo* validation and assessment of NP consistency and stability are needed for clinical use
61	Crisan *et al.*[61]	Not stated	AgNP	*E. coli*, *P. aeruginosa*, and *K. pneumoniae*	*In vitro* experimental study	Showed broad antibacterial activity; combining antibiotics with AgNPs greatly enhanced inhibition zones	Disrupted membranes, triggered ROS, and impaired protein and DNA function	Boosted efficacy against MDR and pandrug-resistant strains, showed broad Gram-negative activity, and may lower required antibiotic doses	Limited to *in vitro* studies; *in vivo* validation, synthesis standardization, and scalability need further research for clinical use
62	Nawaz *et al.*[62]	China	Ag-doped and Zn-doped silica NPs	Periodontitis-causing microbes	*In vitro* experimental study	Showed the strongest antibacterial effect, inhibiting growth and biofilm formation	Disrupted membranes, generated ROS, and hindered enzymes and DNA replication	The mesoporous structure boosted antimicrobial action, limited resistance, and enabled targeted, sustained delivery beyond periodontitis	Limited to *in vitro* data with unspecified pathogens; *in vivo* validation, biocompatibility, and long-term safety still unconfirmed
63	Hayee *et al.*[63]	Pakistan	Levofloxacin-loaded polymeric NPs	*K. pneumoniae*	*In vitro* experimental study	Chitosan-III and poly-lactic-co-glycolic acid-I NPs improved Gram-negative antibacterial activity, offering sustained release and stable formulation	Improved delivery, stability, controlled release, and site-specific retention	Boosts efficacy, stability, and bioavailability, with low hemotoxicity and potential to restore sensitivity in resistant strains	Limited to *in vitro* tests; red blood cell binding by chitosan NPs may hinder delivery. *In vivo* studies and long-term safety evaluation are needed for clinical use

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
64	Mishra *et al.*[64]	India	AgNPs	*K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *Enterobacter* spp.	*In vitro* study	Effectively targeted multiple antibiotic resistance ESKAPE pathogens, showing synergy with antibiotics and reducing required doses by up to 32-fold	Disrupted bacterial membranes, causing cytoplasmic leakage	Showed increased effectiveness against resistant pathogens, biofilm removal potential, and cancer cell selectivity for targeted therapy	Exhibited high cytotoxicity to melanoma cells, raising therapeutic concerns; limited to *in vitro* testing without *in vivo* validation
65	Wang *et al.*[65]	China	Epigallocatechingallate–ferric complex NPs	*E. coli*	*In vitro* and *in vivo* experimental study	Showed strong photothermal antibacterial and anti-biofilm effects, with broad activity against bacteria like *E. coli* under near-infrared light	Enhanced killing via photothermal conversion, with stable antimicrobial action; ferric ions aided biofilm disruption under light	Light-triggered photothermal action boosts antibacterial effects, aids wound healing, reduces antibiotic use, and combats resistance	Photothermal activation in deep tissue infections may be limited by the need for an external light source (near infrared)
66	Campos *et al.*[66]	Brazil	Zein NPs coated with chitosan	*P. aeruginosa* and *Klebsiella pneumoniae*	*In vitro* experimental study	Enabled controlled drug release in gastric and intestinal conditions, effectively eliminating *P. aeruginosa* and *K. pneumoniae* biofilms	Controlled antibiotic release sustained therapeutic levels, while combining ceftazidime and tobramycin boosted efficacy against resistant and biofilm-related infections	Antibiotic co-delivery and sustained release improved efficacy, enhanced biofilm penetration, and tackled resistance mechanisms	*In vitro* only; *in vivo* efficacy, safety, scalability, and regulatory hurdles remain unaddressed
67	Mendez-Pfeiffer *et al.*[67]	Mexico	Thiolchitosan-coated AgNPs	Uropathogenic *E. coli* (UPEC)	*In vitro*	Showed strong antibacterial, antibiofilm, and anti-adherence activity against UPEC	Disrupted membranes and downregulated *fimH* and *PapG*, reducing bacterial adherence and enhancing antimicrobial effects	Effectively inhibited biofilms and adhesion by targeting virulence factors, with strong antimicrobial action at low doses via Ag–thiolated chitosan synergy	The mechanism of adherence inhibition remains unclear; *in vitro*/*in silico* findings require *in vivo* validation and toxicity studies for clinical use
68	Ferreyra Maillard *et al.*[68]	Argentina	AgNP	*E. coli*	*In vitro*	Induced high ROS in bacteria and disrupted membranes in *E. coli*.	Induced oxidative stress via ROS and disrupted *E. coli* membrane integrity	Effectively targeted Gram-negative bacteria, including resistant strains, via ROS induction and membrane disruption	Limited to *in vitro* analysis; lacks *in vivo* data, cytotoxicity assessment, and selectivity evaluation between bacteria and host cells

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
69	Konda *et al.*[69]	India	Green-synthesized AgNP	*P. aeruginosa*	*In vitro*	Caused major metabolic disruptions in *P. aeruginosa*, impacting cell wall, energy, nucleotide pathways, and the tricarboxylic acid cycle in response to NP exposure	Disrupted essential metabolic pathways, including cell wall synthesis and energy production	An eco-friendly alternative and systems-level insight into bacterial responses, supporting targeted antimicrobial design	Restricted to *in vitro*; *in vivo* relevance and toxicity are yet to be assessed
70	Almutleb *et al.*[70]	Saudi Arabia	NilavembuChoornam–AuNPs (NC–GNPs)	*K. pneumoniae* and *P. aeruginosa*	*In vitro*	Showed strong antibacterial action against resistant strains, outperforming NC extract, by inhibiting biofilms and causing nuclear shrinkage, cell wall, and chromosomal damage	Disrupt bacterial cell wall and membrane integrity	Exhibited strong antibacterial activity against resistant strains, surpassing NC extract, by blocking biofilms and inducing nuclear shrinkage, cell wall, and chromosomal damage	Limited to *in vitro* assays; long-term ocular biocompatibility and NC–GNP toxicity remain unassessed.
71	Sathiyaseelan *et al.*[71]	China	Chitosan-fabricated tellurium NPs	*E. coli* and *S. enteric*	*In vitro*	Showed strong antibacterial, antibiofilm, and antioxidant activity, with selective toxicity toward A549 and PC3 cancer cells over normal cells	The ion release in acidic conditions likely boosts antimicrobial action via membrane disruption and oxidative stress	Displayed antibacterial and antioxidant effects, selective cancer cell toxicity, and biocompatible delivery via a chitosan matrix	Limited to *in vitro* studies; *in vivo* efficacy, toxicity, and long-term safety remain unassessed
72	Ankudze *et al.*[72]	Ghana	Biogenic AgNPs	*E. coli*	*In vitro* study	Sunlight-synthesized, showed strong antimicrobial and anti-biofilm activity against *E. coli* at 3.9 μg/mL, with variable synergistic to antagonistic effects	Disruption of cell walls and membranes triggers ROS production, causing oxidative stress, blocking biofilms, and disrupting quorum sensing	Enhances antimicrobial and antibiofilm effects, fights resistance, acts broadly against microbes, and synthesizes quickly under sunlight	Study was conducted *in vitro*
73	Rahman *et al.*[73]	India	Chitosan NPs	*E. coli*	*In vitro*	Showed strong antibacterial activity with a 36±0.8 mm inhibition zone against streptomycin-resistant *E. coli*	Exerted antibacterial action by damaging bacterial membranes and inhibiting growth	Biodegradable, biocompatible, and broad-spectrum against MDR bacteria, offering an eco-friendly alternative to synthetic antibiotics	The study lacked assessment of chitosan NPs' long-term stability and real-world *in vivo* effectiveness

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
74	Allend *et al.*[74]	Brazil	Bio-AgNP	*A. baumannii*	*In vitro*	Showed antibacterial activity (MIC: 0.46–1.87 μg/mL) and synergized with meropenem, reducing its MIC 4–8-fold	Displayed antibacterial activity by disrupting bacterial membranes and causing cellular leakage	Bio-AgNPs and meropenem show promise against carbapenem-resistant *A. baumannii*, boosting antibacterial action and blocking biofilms	Tested *in vitro*; further *in vivo* studies on resistance and Bio-AgNP toxicity are needed for broader conclusions
75	Rajchakit *et al.*[75]	New Zealand	AuNPs–lipopeptide NPs	*E. coli*, *P. aeruginosa*, and *A. baumannii*	*In vivo*	Strong antimicrobial activity with MICs of 0.13–1.25 μM against clinical isolates	Targeted and disrupted bacterial membranes, inhibiting growth and biofilm formation	Enhances enzymatic stability, boosting antimicrobial activity and degradation resistance	The long-term stability and clinical toxicity of the conjugates require further study before broad application
76	Masoudi *et al.*[76]	Iran	TiO_2NPs and ZnONPs	*A. baumannii* and *E. coli* (MDR isolates)	*In vitro* study	Showed strong antioxidant activity and biofilm inhibition of *A. baumannii* and *E. coli*, with ZnONPs especially effective against *E. coli*	Boosted amoxicillin-clavulanic acid's effects by disrupting membranes and blocking biofilms	Showed strong synergy with antibiotics, reducing their MICs up to eightfold	Limited to *in vitro* studies; *in vivo* research is needed to confirm NP safety and efficacy
77	SamadiAfshar *et al.*[77]	Iran	AgNP	*E. coli*	*In vitro*	Exhibited notable antibacterial activity against *E. coli* (21.51±0.61mm zone; MIC 18 μg/mL). Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of proteins and phenols as stabilizers	Disrupted bacterial membranes and interacted with cellular components via silver ions, inhibiting growth or causing death	Showed a plant-based, affordable alternative effective against urinary tract infection pathogens, including resistant strains, with antifungal benefits	Conducted only *in vitro*; *in vivo* studies are needed to evaluate safety, efficacy, pharmacokinetics, and toxicity before clinical use
78	Liu *et al.*[78]	China	Copper sulfide (CuS) NPs	*E. coli*	*In vitro*	Protoporphyrin, CuSNPs, phenylboric acid, and bovine serum albumin complexes exhibited strong singlet-oxygen generation, high photothermal efficiency, and effective targeting of *E. coli*	Convert light into localized heat, killing bacteria	Dual photodynamic and photothermal mechanisms; enhanced bacterial killing and lowered resistance risk	The study was conducted only *in vitro*
79	El Fadl *et al.*[79]	Egypt	Metal oxide NPs	*P. mirabilis*, *P. aeruginosa*	*In vitro* experimental study	Avoided aggregation and improved bacterial interaction via a porous, water-retaining hydrogel matrix	The hydrogel's water content and metal oxides boosted ROS production and bacterial contact, enhancing antimicrobial efficacy	Hydrogel–NP synergy boosted antibacterial action, while preventing aggregation improved stability and efficacy	Needs optimization for clinical use, including cytotoxicity testing and formulation stability

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
80	Karimitabar *et al.*[80]	Iran	AgNP	*E. coli*	*In vitro*	Exhibited superior antibacterial activity compared to AgNP or propolis alone	Drug-degrading enzymes, poor drug penetration, and protective surface proteins	The compound boosts antibacterial efficacy over Ag or propolis alone, offering broad-spectrum	Limited to *in vitro* agar diffusion, the study may underestimate propolis effectiveness in such assays
81	Younis *et al.*[81]	Czech Republic	TiO_2NPs	*E. coli*	*In vitro* study	Modified TiO_2NPs with geraniol showed effective antibacterial activity against *E. coli*	Generated ROS penetrated bacterial walls, damaging components and causing cell death	Effectively targets resistant strains and reduces antibiotic-resistant biofilm formation	Study is limited to *in vitro*
82	Messaoudi *et al.*[82]	Algeria	Ag carbonate NPs	*K. pneumoniae* (kp6 strain)	*In vitro* study	Showed broad-spectrum antimicrobial activity, inhibiting growth at just 1.17 µg/mL	Acts by inhibiting biofilm formation, as shown against *K. pneumoniae*	Effectively targets biofilms and shows broad-spectrum activity against diverse pathogens	*In vitro* only, no *in vivo* data
83	Alipour-Khezri *et al.*[83]	Iran	ZnONP	*P. aeruginosa*	Experimental *in vitro* study	Combined with bacteriophages, it greatly reduced *P. aeruginosa* biofilms and improved bacterial killing over individual treatments	Suppressed *lasI* expression, disrupting quorum sensing and biofilms, while phages lysed bacteria and boosted biofilm clearance	Bypasses antibiotic resistance, eco-friendly, targets biofilms effectively, and may lower cytotoxicity versus ZnONPs alone	Conducted *in vitro*; *in vivo* efficacy, safety, and stability of the ZnONP–phage combo need validation
84	Pacheco *et al.*[84]	Columbia	Phytic acid cross-linked chitosan NPs loaded with colistin.	*P. aeruginosa*	Experimental *in vitro* study	Lowered colistin MICs for both sensitive and XDR *P. aeruginosa*, enhancing antibacterial efficacy	Colistin's membrane disruption and chitosan's improved delivery via electrostatic interactions with bacteria	Enhances colistin efficacy, enables lower dosing, reduces toxicity and resistance, and restores activity against resistant strains	Limited to *in vitro* testing; *in vivo* validation of safety, pharmacodynamics, and long-term efficacy is needed
85	Almowallad and Alqahtani[85]	Saudi Arabia	Chitosan NPs	*P. aeruginosa*	Experimental *in vitro* study	Showed strong antibacterial, antioxidant, and antibiofilm effects, especially against MDR *P. aeruginosa*	Synergistic phytochemicals disrupted membranes, boosted by NP-targeted delivery and sustained release.	Enhanced antibacterial and antioxidant effects against MDR pathogens, with fewer side effects, reduced resistance, and eco-friendly green synthesis	Limited to *in vitro* evaluations; *in vivo* toxicity, pharmacokinetics, and long-term effects require further preclinical study
86	El Megdar *et al.*[86]	Morocco	AgNP	*A. baumannii*	*In vitro* experimental study	Exhibited strong antibacterial activity against multidrug-resistant pathogens	Disrupt bacterial membranes, generate ROS, and interfere with DNA and proteins	Dual antimicrobial and antioxidant effects, target MDR pathogens, and show lower toxicity and resistance risk than traditional antibiotics	*In vitro* study: no *in vivo* or clinical validation

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
87	Celebi *et al.*[87]	Turkey	ZnONP	*K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*	*In vitro* experimental study	Exhibited potent antibacterial activity, while their combination with Zn borate (ZB) resulted in a synergistic effect. The co-administration of ZnO and ZB produced a more pronounced cytotoxic impact than when each was used individually	Antibacterial action involves membrane disruption via electrostatic interaction, Zn^{2+} release, and ROS generation, with ZB boosting efficacy through complementary mechanisms	Outperformed antibiotics with stronger action, lower doses, less toxicity, better stability, and anticancer effects	*In vitro* only; A549 cytotoxicity indicates potential side effects
88	Pourhajibagher *et al.*[88]	Iran	Hypericin NP	*A. baumannii*	Experimental laboratory study	HypNP@D-Tryptophan exhibited synergistic antimicrobial activity against *A. baumannii*	Acted as photo-sonosensitizers	Targeted action, dual activation, reduced resistance risk, biocompatibility, and biofilm penetration	*In vitro* only; single strain tested; no reproducibility; potential *in vivo* toxicity
89	Bayram Sarıipek[89]	Turkey	AgNP	*E. coli*	*In vitro* experimental study	Curcumin–AgNPs (~19.83 nm) in poly(3-hydroxybutyrate)/ chitosan nanofibers improved hydrophilicity and thermal stability and exhibited strong antibacterial activity	Exerted bactericidal effects via membrane disruption and metabolic interference, with curcumin adding synergistic action	Combined curcumin and nanotechnology enhanced antibacterial action and scaffold functionality	Findings were *in vitro* only and lacked *in vivo* validation of efficacy, safety, and wound healing
90	Hussein *et al.*[90]	Iraq	AgNP	*Serratia marcescens*	Experimental study	Exhibited 100% antibacterial activity against *S. marcescens*	Increased ROS generation damaged bacterial DNA, proteins, and membranes	Effective against MDR bacteria with broad-spectrum activity due to its small size	Limited to *in vitro* testing on a single bacterial species; human toxicity of AgNPs remains unassessed
91	Ahmed *et al.*[91]	Egypt	AgNP combined with H_2O_2	MDR extended-spectrum β-lactamase-producing *E. coli* (ESBL-*E. coli*)	Experimental study	Showed complete bacterial inhibition at concentrations of 0.625–5 μg/mL	Disrupts bacterial membranes, increases permeability, and generates ROS, leading to cell death	Requires lower effective doses than conventional antibiotics and poses a reduced risk of promoting antibiotic resistance	Limited to one region and sample type; only *in vitro* results; environmental impact unassessed
92	Fayed *et al.*[92]	United Arab Emirates	Amoxicillin –loaded chitosan NP	*H. pylori*	*In vitro* experimental study	Reduced MIC_{50} against *H. pylori* and effective sustained drug release over 72 h	Exhibited antimicrobial activity against *H. pylori*; encapsulation enhanced efficacy and inhibited resistance via efflux pump suppression	Inulin co-delivery targeted *H. pylori* while preserving gut microbiota balance	Limited to *in vitro*; *in vivo* validation needed for clinical relevance and microbiota impact

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
93	NajeerAhamed *et al.*[93]	India	*Colletotrichum gloeosporioides* AgNPs	*E. coli*	*In vitro* experimental study	Reduced *E. coli* biofilm formation, increased membrane permeability, and triggered oxidative stress, contributing to antibacterial action	Induced oxidative damage, leakage of cell contents, and suppressed CTX-M-15 expression in *E. coli*	Reduces biofilms and resistance by oxidative stress and gene disruption	Study limited to *in vitro*; *in vivo* validation needed for safety and efficacy
94	Albdrawy *et al.*[94]	Saudi Arabia	Chitosan NPs	MDR *Neisseria gonorrhoeae*	Experimental study	Enhanced anti-gonococcal and antibiofilm activity compared to free peptides	Disrupted bacterial membranes via electrostatic interactions	Protected peptides from degradation and enabled sustained antimicrobial release for prolonged activity	Only *in vitro* tests were performed; long-term effects and immune responses remain unassessed
95	Ciftci *et al.*[95]	Turkey	AuNP	*A. baumanii*, *K. pneumoniae*, *E. coli*, *Proteus* spp., *S. marcescens*, and *P. aeruginosa*	Experimental study	Antibiotic coatings were more effective against *E. coli*, *Proteus* spp., and *Serratia marcescens* than pure antibiotics	Prevent resistance by increasing cell permeability, inhibiting efflux pumps, and blocking biofilm formation	Their small size, large surface area, and non-toxic, unstable nature make them versatile for medical applications	Ensure safety to avoid cytotoxicity; validate with *in vivo* studies before clinical use
96	Abdolmasoudi *et al.*[96]	Iran	Selenium NP loaded with ampicillin	*K. pneumoniae*	Descriptive cross-sectional study	Suppressed *OqxB* gene expression, increasing ampicillin susceptibility	Serves as a cofactor for antioxidant enzymes, protecting against free radicals	Reduces antibiotic resistance and enhances targeted antibiotic delivery to bacterial cells	Stability and safety need evaluation due to possible toxicity and environmental impact
97	Huang *et al.*[97]	China	Ceftazidime-decorated AuNPs	Ceftazidime–avibactam-resistant Enterobacteriaceae	Experimental study	Killed resistant strains and strongly inhibited and eradicated biofilms	Increased membrane permeability and ROS generation caused bacterial death	Enhanced ceftazidime delivery and potency with strong activity against MDR and heteroresistant strains	Production scale-up, stability, regulation, and long-term toxicity remain unaddressed
98	Ram *et al.*[98]	India	Nanosilver-conjugated thymol	MDR enteroaggregative *E. coli* (MDR-EAEC)	*In vitro* and *in vivo* experimental study	Demonstrated a lower MIC and MBC (4–16 μg/mL and 8–16 μg/mL, respectively)	Likely due to a nanosilver–thymol synergy that disrupts bacterial membranes	Shows lower MIC/MBC than thymol, better safety, stability, and eco-friendliness, with efficacy like meropenem	Tested only *in vitro* and *in vivo* (larval model); human trials are needed to confirm safety and efficacy
99	Palau *et al.*[99]	Spain	AgNP functionalized with mercaptopoly(ethylene glycol) carboxylic acid and amikacin	MDR and XDR strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*	*In vitro* study	×10 more effective than amikacin alone; killed all MDR/XDR strains	Combines AgNPs and amikacin, with hyperthermia enhancing antibacterial and antibiofilm effects	Outperforms amikacin alone; more stable and, with hyperthermia, boosts antibacterial and antibiofilm action against MDR/XDR strains	Tested only *in vitro*; lacks *in vivo* validation for real-world use

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
100	Adnan *et al.*[100]	Saudi Arabia	AgNP from saponins of *Ajwa dates*	*P. aeruginosa*, *E. coli*, *P. vulgaris*	Experimental laboratory-based study	Showed strong antibacterial and dose-dependent biofilm inhibition	Disrupts bacterial membranes, causing leakage of DNA, RNA, and proteins	Enhanced antimicrobial activity via antibiofilm and antioxidant effects; targets multiple survival pathways like membrane disruption and quorum sensing inhibition	Focused on laboratory strains and *in vitro* tests only; lacked validation with clinical isolates or *in vivo* models
101	Pant *et al.*[101]	United States	Ultrafine bismuth subcarbonate NPs functionalized with polyvinylpyrrolidone	MDR and drug-susceptible *P. aeruginosa*	Experimental study	Broad-spectrum antibacterial activity, effective even against resistant mutants (*P. aeruginosa* resistant to AgNPs, meropenem, ciprofloxacin)	ROS generation, physical membrane disruption observed by SEM	Shows promise for topical or combination therapy with low cytotoxicity (IC_{50} > 40–50 μg/mL) to human dermal fibroblasts and macrophages	Environmental impact, scalability, pharmacokinetics, and long-term effects were not addressed
102	Mandal *et al.*[102]	United States	Chitosan-modified, high-surface-area activated carbon derived from kenaf (KAC) AgNP	*E. coli*	Experimental study	Demonstrated bacterial resistance and is a promising eco-friendly material for bacterial disinfection	Ag^+ interacts with membranes, generates ROS and disrupts cell wall integrity, leading to cell death	KAC offers high surface area for pollutant adsorption; AgNPs add antimicrobial and pollutant-binding effects via surface interaction and ion exchange	The exact antibacterial mechanism of AgNPs remains unclear
103	Elekhnawy *et al.*[103]	Egypt	ZnONPs synthesized by *Cycas circinalis*	*P. mirabilis*	Experimental study involving in *vitro* and *in vivo* assessments.	Showed antibacterial activity, reduced bacterial load and improved survival in infected mice	Disrupted bacterial membrane integrity, leading to bacterial cell death	Showed strong antibacterial activity (MIC 32–128 μg/mL) and better *in vivo* outcomes than standard antibiotics	Lacked long-term safety and toxicity data, particularly regarding systemic use in humans
104	Francis *et al.*[104]	United Arab Emirates	Biogenic CuO, ZnO, and tungsten trioxide (WO_3) NPs	*E. coli*	Experimental laboratory-based study	WO_3, CuO, and ZnO NPs showed >90% antimicrobial activity in combination at low doses—more effective than individual use	Generated ROS, causing oxidative stress and damaging lipids, proteins, and DNA	They outperform conventional agents, effectively targeting diverse Gram-negative bacteria despite complex cell walls	The exact mechanism of WO_3 NPs remains unclear due to limited studies, hindering validation and comparison
105	Giráldez-Pérez *et al.*[105]	Spain	Au@16-mph-16 Au NPs	*E. coli*	Experimental laboratory-based study	Their stability, low toxicity, and biocompatibility make them ideal drug carriers. Combined with Au@16-mph-16, they boost bacterial killing at low doses.	Synergistically enhanced antimicrobial activity beyond individual effects	Synergistic action with high stability, biocompatibility, and targeted drug delivery	Requires further testing against diverse strains; long-term toxicity remains unassessed

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
106	Caballero Gómez *et al.*[106]	Spain	Ag, Au, Zn and Cu NPs	*Pseudomonas* spp.	Experimental study	Combined with ethylenediaminetetraacetic acid (EDTA) or disinfectant formulations based on EDTA, it inhibited both planktonic cells and biofilms at low doses	Ag and ZnO NPs directly damage bacterial membranes, causing content leakage and membrane depolarization	Can be customized for targeted action, are effective at low doses, and disrupt biofilms when combined	Biocompatibility and cytotoxicity of top NP combinations must be assessed before clinical use
107	AbdElrahman *et al.*[107]	India	ZnONP	*Pseudomonas* spp., *E. coli*	Experimental laboratory-based study	Pure, nanoscale, and morphologically distinct; showed strong activity against MDR *Pseudomonas* spp., indicating medical potential	Exert antibacterial action via ROS generation, Zn ion release, and membrane damage through direct contact	Offer eco-friendly, broad-spectrum antibacterial action against MDR pathogens via multiple mechanisms and nanoscale advantages	Limited to *in vitro* data; no toxicity assessment; variable NP size; no comparison with chemical ZnONPs; weak plant extracts alone
108	Ugalde-Arbizu *et al.*[108]	Spain	MSNs functionalized with Ag^{+} and Cu^{2+}	*E. coli*, and *P. aeruginosa*	Experimental laboratory-based study	Demonstrated promising antibacterial and biofilm-inhibitory effects	Combats *P. aeruginosa* via Ag-induced membrane damage and synergistic effects of Ag ions and phenytoin	Provides broad-spectrum, potent activity against MDR strains, enhances biofilm inhibition, supports wound healing, and lowers resistance risk via synergy with phenytoin and controlled release	Ineffective against mature biofilms; *in vivo* validation needed for clinical use.
109	Saleem *et al.*[109]	Pakistan	Antibiotic-conjugated *Alternanthera pungens* and *Trichodesma indicum* CuNPs	*K. pneumoniae*, *Salmonella* Typhimurium	Experimental laboratory-based study	*A. pungens*-derived conjugates of gentamicin showed strong antibacterial activity, DNA protection, and ROS induction; ampicillin-conjugated NPs were mostly ineffective	Synergy between gentamicin and *A. pungens* bioactives, causing oxidative stress, membrane damage, and DNA protection	Eco-friendly synthesis, enhanced MDR activity at low doses via DNA protection and oxidative stress	Ampicillin–CuNPs showed poor efficacy, exhibited moderate hemolysis indicating cytotoxicity, lacked *in vivo* validation, and were tested on limited bacterial strains
110	Aziz *et al.*[110]	Iraq	Binary Cu–cobalt oxide NPs	MDR *K. oxytoca*	Experimental laboratory-based study	Effectively reduced expression of *papC* and *fimH* virulence genes in MDR *K. oxytoca* isolates	Downregulated *papC* and *fimH* virulence genes, reducing adhesion and biofilm formation	Offers advantages over conventional drugs, downregulates virulence genes, enhances outcomes, and potentially reduces treatment time	Small sample size, no *in vivo* validation, and focus solely on *K. oxytoca*, reducing generalizability

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
111	Dove *et al.*[111]	United States	AgNP	*E. coli*, *P. aeruginosa*, *A. baumannii*, *Salmonella* spp., *K. pneumoniae*, and *C. freundii*	Experimental study	Exhibited potent bactericidal activity against MDR bacteria at low MICs	Target bacterial ribosomes, synergize with aminoglycosides via complementary binding, and spare eukaryotic ribosomes	Exhibit broad-spectrum efficacy, low cytotoxicity, and enhance aminoglycoside potency, supporting their use as stand-alone agents or adjuvants	Relied on *in vitro* and *C. elegans* models; lacked mammalian validation, showed motility reduction, and provided no long-term safety or pharmacokinetics data
112	De Almeida *et al.*[112]	Portugal	Surface-enhanced Raman spectroscopy (SERS) with silver nanostars (AgNS)	*A. baumannii*, *K. pneumoniae*	Pilot study	SERS enables rapid identification of *A. baumannii*/*K. pneumoniae* using simple nanostar (AgNS) synthesis and handheld devices for point-of-care use	Detection of biochemical signals enables species and clone-level identification based on structural/metabolic differences such as capsule composition	AgNS–SERS enables rapid (< 30 min), low-cost, label-free species and resistance identification with minimal processing	Pilot study with limited isolates and species; broader validation needed for reproducibility and clinical relevance
113	James and Kumar[113]	India	AuNP from *Aspergillus niger* CJ14 (S2-10)	*K. pneumoniae*, *A. hydrophila*, and *E. coli*	Experimental *in vitro* study	Showed strong antimicrobial and antibiofilm effects	Inhibited colonization and disrupted biofilm structures at various stages	Broad-spectrum, eco-friendly option that targets multiple biofilm stages in drug-resistant bacteria on diverse surfaces	Limited by *in vitro* design and narrow bacterial range, with unvalidated broader and *in vivo* applicability
114	Nemattalab *et al.*[114]	Iran	Solid lipid NPs (SLNs)	XDR *E. coli*	Experimental study	Cinnamon oil (CO)-loaded solid lipid NPs (CO-SLN) showed significantly enhanced antibacterial and anti-biofilm activity	Enhanced CO delivery and uptake, boosting antibacterial and antibiofilm efficacy	Improved antibacterial potency, biofilm inhibition, and cellular penetration at lower dose	*In vitro* only, limited to a few *E. coli* strains, no *in vivo* or broad-spectrum validation.
115	Asif *et al.*[115]	India	ZnONP	*E. coli*	*In vitro* experimental study	Showed strong antibiofilm activity along with significant biofilm disruption and bacterial viability reduction	Bacterial membrane permeability, ROS generation, and disrupted membrane integrity	Green, eco-friendly synthesis with strong penetration and biofilm disruption, effective against resilient biofilms, promising alternatives to conventional antibiotics	*In vitro* only and lacks *in vivo* validation for clinical applicability and biosafety
116	Abdul-Jabbar *et al.*[116]	Iraq	AgNP	*B. cereus* and *P. mirabilis*	Laboratory-based experimental study	Exhibited antibacterial and antioxidant activity, enhanced further when combined with lincomycin	Inhibit bacterial growth via membrane disruption	Showed enhanced antibacterial activity and potential to reduce bacterial resistance	Limited by the scope of pathogens studied

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
117	Prasastha Ram *et al.*[117]	India	AgNP and cinnamaldehyde-entrapped Ag (AgC) NPs	MDR-EAEC	Experimental *in vitro* and *in vivo* study	The AgNPs–AgC combo exhibited strong, stable, and safe antimicrobial activity	Cinnamaldehyde targets bacterial membranes; AgNPs cause protein denaturation and cell damage	Cinnamaldehyde–AgNPs combo boosts activity against MDR-EAEC, enhances stability, reduces toxicity, enables targeted delivery, and improves bioavailability	AgCNPs may agglomerate, reducing efficacy; they exhibit moderate cytotoxicity at high doses and strain-specific activity
118	Kang *et al.*[118]	South Korea	C2-AuNPs and C2-ZnONPs	*K. pneumoniae*, *E. coli*, *L. monocytogenes*, *P. aeruginosa*, *V. vulnificus*	Experimental study	C2-AuNPs inhibited *P. aeruginosa* biofilm; C2-ZnONPs inhibited both biofilm formation and planktonic growth	Disrupt bacterial membranes, inhibit biofilm-forming genes, and ROS generation	Offer superior biofilm inhibition, multifunctionality, and biocompatibility, making them effective against chronic infections	Biofilm inhibition was concentration-dependent, showing variable efficacy across strains
119	More *et al.*[119]	Italy	Bimetallic NPs (AgPtNPs) and monometallic NPs (AgNPs and PtNPs)	*E. coli* (ATCC 11229), *K. pneumoniae* (ATCC 10031)	Evaluation study	Well-synthesized, stable bimetallic NPs outperformed AgNPs and PtNPs, showing partial bactericidal and bacteriostatic effects	AgPtNPs likely act by destabilizing bacterial membranes, forming pores, and disrupting cell integrity	Showed strong antimicrobial efficacy, low MICs, and synergism with antibiotics, offering a promising alternative to traditional agents	Effective against various strains, but less so for some MDR strains; long-term stability and environmental impact remain unassessed
120	Sivaraj *et al.*[120]	India	β-tricalcium phosphate/f-multi-walled carbon nanotube (MWCNT; MTCP), Zn-substituted β-tricalcium phosphate/f-MWCNT (ZMTCP), and Zn and magnesium (Mg)-substituted β-tricalcium phosphate/f-MWCNT (ZMTCP1)	*A. baumannii*	*In vitro* study	Increasing Zn concentration reduced crystallite size and altered biological activity; FTIR confirmed Zn and Mg substitution in the tricalcium phosphate structure	Mg and Zn substitution in β-tricalcium phosphate enhances bone regeneration, integration, and cell proliferation, supporting biomedical use	ZMTCP and ZMTCP1 nanocomposites improve implant coatings by enhancing biocompatibility, reducing bacterial adhesion, and promoting osteointegration	There is a need for further optimization of nanocomposite size and performance
121	Darwish and Salama[121]	Jordan	Tryasine–AgNPs	*E. coli*, ESBL *E. coli* (ATCC BAA-3054)	Experimental laboratory-based antimicrobial study	Effective against *E. coli*, including ESBL strains, with enhanced antibacterial and bactericidal activity, low hemolysis, and minimal cytotoxicity	Increases bacterial permeability, confirming a bactericidal and not just inhibitory effect	Enhanced antibacterial activity against XDR strains offering a safer alternative to conventional antibiotics	High cost and potential cytotoxicity at higher doses remain concerns

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
122	Khleifat *et al.*[122]	Jordan	AgNP	*E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, *Shigella* spp., *P. aeruginosa*	Experimental study	Showed broad-spectrum antibacterial activity, though *P. aeruginosa* was less susceptible. Combining AgNPs with antibiotics enhanced efficacy against resistant *E. coli* and *K. pneumoniae*	Nicotinamide adenine dinucleotide-dependent AgNP formation disrupts bacterial electron transport, reduces ATP levels, damages membranes, inhibits DNA unwinding, and induces oxidative stress, leading to cell death	Broad-spectrum action, boosts antibiotic efficacy, and helps combat resistance.	Broad antibacterial activity observed, but limited efficacy against *P. aeruginosa*; variable synergy with AgNPs; mechanism unclear, requiring further study
123	Alotaibi *et al.*[123]	Saudi Arabia	AgNP	*E. coli* (ATCC 25922), *A. baumannii* (ATCC BAA-747), *K. pneumoniae* (ATCC 13883 + three antimicrobial-resistant, clinical isolates), *P. aeruginosa* (ATCC 27853)	*In vitro* study	Demonstrated strong antimicrobial activity (MIC: 16–128 µg/mL) and synergism with select antibiotics, enhancing potency	Disrupt cell walls, generate ROS, and release silver ions upon internalization due to wall structure and charge	Antibiotics effectively target AMR strains such as *K. pneumoniae*, show synergy with various antibiotics, have broad-spectrum activity, and exhibit sustained effects from good physicochemical stability	Possible aggregation in biological media, limited long-term stability, and variable efficacy from size, coating, and surface charge differences
124	Qurbani *et al.*[124]	Iraq	AgNP	*P. aeruginosa*, *Salmonella* spp., *Shigella* spp., *E. coli*, *Enterobacter* spp., *K. pneumoniae*, *B. cereus*	Experimental study	Showed strong antibacterial activity against all tested MDR pathogens, with lower MIC/MBC values than tetracycline and azithromycin	Disrupted bacterial membranes, interacted with functional groups, and possibly caused oxidative stress or inhibited cellular processes	Shows lower effective doses than tetracycline and azithromycin, broad-spectrum activity against MDR pathogens, and is biosynthesized via eco-friendly bacterial methods	Potential eukaryotic toxicity, size variability (10–80 nm), and need for *in vivo* safety studies
125	Ghani *et al.*[125]	Iran	Crystalline AgNP	*Bacillus cereus* and *Escherichia coli*	Experimental study	Showed antibacterial activity, with stronger effects at higher concentrations	Adhesion, penetration, ROS generation, signal disruption, and bacterial protein binding, leading to growth inhibition or death	Eco-friendly, non-toxic, broad-spectrum antimicrobial with multi-target action, comparable to standard antibiotics, and cost-effective	No antibacterial effect at 0.5 mM AgNP; limited spectrum tested; compared only *in vitro* to antibiotics; stability and long-term safety unexamined

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Table S1. (*Continued*)

Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
126	Tiri *et al.*[126]	Turkey	Ag–Pt bimetallic NPs using propolis extract	*E. coli*, *B. subtilis*, *K. pneumoniae*, *S. marcescens*	Experimental study	Showed strong antibacterial activity, high catalytic efficiency for hydrogen production, and enhanced catalyst stability and reusability	Disrupt bacterial membranes, interfere with metabolism, and catalyze sodium borohydride hydrolysis to produce hydrogen gas	Enhanced antibacterial and catalytic efficiency, exhibiting reusability and stability, and broad-spectrum antibacterial activity	*In vitro* only; lacks *in vivo* validation, toxicity, long-term stability, biocompatibility, and scale-up or cost-effectiveness data
127	Scandorieiro *et al.*[127]	Brazil	Biogenically synthesized AgNP (bioAgNP)	*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*	Experimental laboratory study	Show strong antibacterial effects against reference and MDR bacteria, lowering MICs and rapidly killing bacteria when combined with oregano compounds	Disrupt membranes, generate ROS, and damage DNA and proteins, leading to cell leakage	Effective against MDR strains with rapid killing, using oregano-derived agents, and eco-friendly synthesis to lower doses and toxicity	No clear MIC difference between Ag nitrate and bioAgNPs; some combos slightly reduced doses without significance; mechanism unclear
128	Hazime *et al.*[128]	France	Alginate NPs	*E. coli* ATCC *8739* and *E. coli 184*	*In vitro* experimental study	Effectively adsorbed dermaseptin B2 showed antibacterial activity against colistin-resistant *E. coli*, remained stable under simulated gastrointestinal tract conditions, and exhibited low cytotoxicity and hemolysis	Targeted delivery, disrupt bacterial membranes, and small molecules like menthol/lactic acid	Biocompatible and stable, effectively combats colistin-resistant *E. coli*, ensuring sustained release and reduced toxicity, and enhancing stability for oral delivery	Needs *in vivo* validation, with potential peptide loss during dialysis and no long-term toxicity data or pharmacokinetics provided
129	Fan *et al.*[129]	USA	Chitosan-based NPs	*E. coli* (multiple strains), *P. azotoformans*, and *K. pneumoniae*	Experimental laboratory study	Combining β-lactams with β-lactamase inhibitors lowers MICs against ESBL bacteria, restoring susceptibility; chitosan NPs further boost this antimicrobial effect	β-lactams are weakened by β-lactamases; inhibitors and chitosan NPs restore and boost their effect	Lower MICs, combat ESBL strains, reduce resistance, and enable stable, versatile antibiotic delivery with added antimicrobial action	Clavulanate and tazobactam outperformed sulbactam in restoring susceptibility. Higher sodium sulfate levels reduced loading efficiency
130	Muneer *et al.*[130]	Pakistan	Mg oxide NPs	*E. coli*	Experimental study	Increased snail mortality and high *E. coli* prevalence in Houbara bustard birds, indicating strong antimicrobial effectiveness	Mg^{2+} ions released, ROS generated; when combined with antibiotics, they boost drug delivery and bactericidal effects	Improve antimicrobial action, lower resistance, and increase stability	Snail deaths and tissue damage suggest environmental toxicity; High MIC variability shows inconsistent antimicrobial effects; *E. coli* remains resistant despite combined treatments

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Serial number	Citation	Country	Nanomaterials used	Gram-negative pathogen/bacteria targeted	Study type	Key findings	Mechanism of action of the nanoparticles	Advantages of nanoparticles over conventional agents	Limitations
131	Piri *et al.*[131]	Iran	Fe oxide NP	*P. aeruginosa*	Experimental laboratory study	Exhibited strong antibacterial effects by inhibiting growth and suppressing *CTX-M* gene expression, achieving near-complete bacteriostasis in 24 h	Reduces antibiotic resistance in P. aeruginosa dose-dependently by inhibiting *bla-CTX-M* gene expression.	Effectively treats MDR strains, including *CTX-M*, via a non-specific mechanism, offering a novel therapy for resistant infections, especially in burn patients	*In vitro* only; lacks clinical validation and data on long-term toxicity, biocompatibility, and environmental or cellular safety
132	Kotb *et al.*[132]	Egypt	ZnONPs	MDR Enterobacterales	Experimental laboratory Study	Showed strong antimicrobial and bactericidal effects combined with 6-pentyl-α-pyrone lactone	Generated ROS, damaged membranes, and released Zn^{2+} ions	Effective against MDR isolates and potential synergy to overcome integrons and high multiple antibiotic resistance indices	Lacks *in vivo* validation, limited lactone efficacy alone, requires further safety and pharmacokinetic studies of ZnONPs and fungal metabolites
133	Al-nemrawi *et al.*[133]	Jordan	ZnONPs	*E. coli*	Experimental *in vitro* study	Coated NPs demonstrated enhanced antibacterial activity, improved tobramycin (TOB)–chitosan release and efficacy, and decreased the MIC compared to uncoated NPs	TOB blocks protein synthesis by binding 30S and 50S ribosomal subunits; ZnONPs produce ROS that damage membranes and induce oxidative stress.	The sustained drug release, enhanced antibacterial activity through synergistic effects of TOB and ZnO, and its potential to overcome antibiotic resistance	*In vitro* only; lacks *in vivo* validation. Larger particle size and uneven ZnO coating may reduce bioavailability and uniformity
134	Parvane *et al.*[134]	Iran	Poly (lactic-co-glycolic acid) (PLGA) NPs	Enteropathogenic *E. coli* (EPEC) and enterohaemorrhagic *E. coli* (EHEC)	Experimental (*in vitro* and *in vivo* animal model)	Reduced organ bacterial burden and 80% and 60% protection against lethal doses of EPEC and EHEC, respectively	Gradual antigen release from PLGA NPs	Cross-protective vaccine potential, controlled sustained antigen release, and dual humoral and cellular immune activation	Oral/mucosal immunization had not been tested, and suitable EHEC/EPEC mouse models were lacking
135	Mohammad *et al.*[135]	Iraq	Fe NP	*P. aeruginosa*	Experimental	Showed dose-dependent antimicrobial activity against *P. aeruginosa*	Interact electrostatically with negatively charged bacterial membranes, forming pits and disrupting cell wall integrity.	Green, eco-friendly synthesis method; effective against antibiotic-resistant strains	No *in vivo* studies performed
136	Khattak *et al.*[136]	Pakistan	AgNP synthesized using leaf extracts	MDR and non-MDR microorganisms	Experimental	Showed higher antibacterial activity (27.75 mm zone of inhibition) than stem-based ones (24.33 mm)	Disruption of bacterial membranes, generation of ROS, and interactions with DNA/proteins	Effective against MDR pathogens and utilizes natural phytochemicals to enhance antimicrobial activity	Long-term toxicity, stability, and dosage optimization not assessed

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Table S1. (*Continued*)

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137	Alshammari *et al.*[137]	Saudi Arabia	AuNP (GNPs)	*E. coli*, *S. abony*, *K. pneumoniae*	Experimental	Ceftriaxone-loaded-GNPs showed ×2 enhanced efficacy compared to pure ceftriaxone	Improves delivery, increases cell wall permeability, and may bypass β-lactamase resistance by concentrating antibiotics at target sites	Enhanced efficacy at lower doses, sustained release, and effectiveness against β-lactamase-related resistance.	Lacks *in vivo* validation; long-term safety and human-specific mechanisms remain unclear
138	Malá *et al.*[138]	Czech Republic	AgNPs	ESBL-producing *K. pneumoniae* (ESBL-KP), Gram-negative bacteria	Experimental (*in vivo*)	Both TMPyP (photosensitizer) and AgNPs individually inhibited methicillin-resistant *Staphylococcus aureus* and ESBL-KP	Disrupted membranes, interacted with DNA respiratory enzymes	Effective against resistant pathogens with low toxicity and targeted photodynamic action.	Photosensitizer efficacy depends on bacterial type and light fluence, requiring precise timing and dose optimization
139	Morshedtalab *et al.*[139]	Iran	Zn sulfide NPs	*A. baumannii*	Experimental (*in vitro*)	Showed dose-dependent antibacterial activity	Generate ROS and disrupt bacterial membranes and protein functions	Effective against antibiotic-resistant strains and an alternative to failing conventional antibiotics and potential low toxicity	No *in vivo* validation
140	Majeed and Wadee[140]	Iran	Nickel ferrite NPs	*P. aeruginosa*	Experimental	Effectively inhibited MDR *P. aeruginosa*; nickel NPs-antibiotic combo accelerated healing in mice	Damage membranes, inhibit enzymes, denature ribosomes, and interfere with DNA replication.	Effective against highly drug-resistant pathogens and enhances antibiotic efficacy, supporting use in burn wound treatment.	No human clinical trial yet

Abbreviations: *P. aeruginosa*: *Pseudomonas aeruginosa*; *A. baumannii*: *Acinetobacter baumannii*; *E. coli*: *Escherichia coli*; *K. pneumonia*: *Klebsiella pneumoniae*; *B. licheniformis*: *Bacillus licheniformis*; *P. mirabilis*: *Proteus mirabilis*; *E. aerogenes*: *Enterobacter aerogenes*; *S. enteritidis*: *Salmonella enteritidis*; *T. denticola*: *Treponema denticola*; *T. forsythia*: *Tannerella forsythia*; *K. variicola*: *Klebsiella variicola*; *A. hydrophila*: *Aeromonas hydrophila*; ATCC: American Type Culture Collection.

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