



A Mini Review of Antibacterial Properties of ZnO Nanoparticles

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The development of antibiotic resistance of bacteria is one of the most pressing problems in world health care. One of the promising ways to overcome microbial resistance to antibiotics is the use of metal nanoparticles and their oxides. In particular, numerous studies have shown the high antibacterial potential of zinc oxide nanoparticles (ZnO-NP) in relation to gram-positive and gram-negative bacteria. This mini-review includes an analysis of the results of studies in recent years aimed at studying the antibacterial activity of nanoparticles based on zinc oxide. The dependence of the antibacterial effect on the size of the applied nanoparticles in relation to *E. coli* and *S. aureus* is given. The influence of various ways of synthesis of zinc oxide nanoparticles and the main types of modifications of NP-ZnO to increase the antibacterial efficiency are also considered.

Keywords: nanoparticles, zinc oxide, antibiotics, antibacterial, bacteriostatic, bactericidal, fungicidal, green synthesis

INTRODUCTION

Today, antibiotics are the “gold standard” in treatment of many bacterial infections [1, 2]. However, microorganisms can develop antibiotic resistance. The majority of pathogenic microorganisms have an ability to develop resistance to at least some antimicrobial agents [3]. Antibiotic resistance in bacteria is achieved by several mechanisms: prevention of drug penetration into a cell [4, 5], changes in an antibiotic target [6, 7], enzymatic inactivation of antibiotics [8], active excretion of an antibiotic from a cell [4] and so on.

According to the data of the World Health Organization (WHO), lower respiratory infections and gastrointestinal infections are among the top ten factors of morbidity and mortality [9]. Appearance of antibiotic resistant strains significantly increased the number of deaths and severity of bacterial infections. Deaths of patients due to antibiotic resistant bacterial strains exceed the total number of global deaths due to cancer and *diabetes mellitus* [10, 11]. Despite the significant quantity of available antibiotics, resistance to almost all of them was confirmed. Antibiotic resistance emerges shortly after a new drug is approved for use [3, 12]. The indicated events urged WHO to endorse the Global action plan on antimicrobial resistance in 2015 [13]. Secondary bacterial infections can be a cause of increased lethality among patients in intensive care; in particular, bacterial co-infection and secondary infection are found in patients with COVID-19 [14, 15]. All above mentioned make a search for new antimicrobial preparations a high priority task of public health in the world.

The number of scientific publications devoted to a search for new antimicrobial compounds is about 99000 only in 2018–2020; 5900 of them are devoted to a search for antibacterial compounds based on metal compounds [16].

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Specialty section:

This article was submitted to
Physical Chemistry and
Chemical Physics,
a section of the journal
Frontiers in Physics

Received: 15 December 2020

Accepted: 19 January 2021

Published: 11 March 2021

Citation:

Gudkov SV, Burmistrov DE, Serov DA,
Rebezov MB, Semenova AA and
Lisitsyn AB (2021) A Mini Review of
Antibacterial Properties of
ZnO Nanoparticles.
Front. Phys. 9:641481.
doi: 10.3389/fphy.2021.641481

Humans have been used antimicrobial properties of several metals and their ions since ancient times. For example, utensils from Cu and Ag were used in ancient Persia, Rome and Egypt [17]. It is known today that a wide range of metals has the antimicrobial activity: Ag, Al, As, Cd, Co, Cr, Cu, Fe, Ga, Hg, Mo, Mn, Ni, Pb, Sb, Te, Zn [18–20].

The basis of the antimicrobial activity of metals is an ability of metal ions to inhibit enzymes [21, 22], facilitate generation of reactive oxygen species (the Fenton reaction) [23], cause the damage of cell membranes [24], prevent uptake of vitally important microelements by microbes [25]; moreover, several metals can exert the direct genotoxic activity [26–28].

The use of nanoparticles based on metals and their oxides is of great interest. One of the well-studied metals affecting biological objects is zinc (Zn) and its oxide (ZnO). Zinc is an active element and exhibits strong reduction properties. It can easily oxidize to form zinc oxide. Zinc plays an important role in the human body, since it is one of the most important trace elements [29]. Zinc is found in all tissues of the human body, with the highest concentration found in myocytes (85% of the total zinc content in the body) [30]. Zinc has been shown to be critical for the proper functioning of a large number of macromolecules and enzymes, where it plays both a catalytic (coenzyme) and structural role. In turn, structures called Zincfinger provide a unique scaffold that allows protein subdomains to interact with either DNA or other proteins [31].

Zinc is also essential for the functioning of metalloproteins. Although zinc is considered relatively non-toxic, there is growing evidence that free zinc ions can cause negative effects on cells. To assess the toxicity of a test substance *in vitro*, animal cell cultures are usually used. It is known that nerve cells are the most sensitive to exogenous influences [32–34]. It has been reported that exposure to zinc ions leads to neuronal degradation [35]. To eliminate the cytotoxic effect, zinc cations are bound with bioactive ligands (for example, proteins) and zinc oxide nanoparticles are synthesized. Nanostructured ZnO can have various morphological forms and properties.

At present, there is a growing interest to nanoparticles of metals and metal oxides as compounds with antibacterial potential: Ag [36, 37], Au [38], ZnO [10], TiO₂ [39, 40], CuO [41, 42], Fe₂O₃ [43, 44]. ZnO has many applications in engineering and medicine. In engineering, ZnO nanoparticles are used in solar cells [45, 46], gas sensors, in particular, sensors for Liquefied petroleum gas (LPG) and EtOH [47], chemical sensors and biosensors, in LEDs, photodetectors [48, 49]. In biology and medicine, the cytostatic activity of ZnO nanoparticles (ZnO-NPs) against cancer cells [50], antimicrobial and fungicidal activities [51, 52], anti-inflammatory activity [53, 54], ability to accelerate wound healing [55], a possibility to use in bioimaging due to chemiluminescent properties of nanoparticles [56, 57], antidiabetic properties [58, 59] are of great interest.

ZnO nanoparticles have several advantages: high antibacterial effectiveness at low concentrations (0.16–5.00 mmol/L), activity against a wide range of strains, relatively low cost [43, 51, 60]. ZnO nanoparticles are synthesized by the physio-chemical sol-gel method from zinc salts [43, 61], sol-gel combustion method [62], solochemical method [63], chemical synthesis at low

temperatures [64] and mechanical method [65]. In several cases, stabilizing agents, for example, chitosan are added [66, 67].

The mechanisms of action of zinc oxide nanoparticles can be reduced to the following: disruption of the cell membrane [68, 69], binding to proteins and DNA, generation of reactive oxygen species (ROS) [10, 70, 71], disturbance of the processes of bacterial DNA amplification, alteration (more often, down-regulation) of expression in a wide range of genes [72]. The direct bactericidal action of ZnO nanoparticles against both gram-negative and gram-positive bacteria and fungi was shown [73, 66, 74].

Nanoparticles of a number of metal oxides lead to the production of ROS upon interaction with bacteria [75]. The metal ions released by the nanoparticles affect the respiratory chain and inhibit some enzymes. This leads to the formation and accumulation of singlet oxygen, hydroxyl radical, hydrogen peroxide, superoxide anions, and other ROS. ROS can cause damage to the internal components of bacteria, such as proteins and DNA [76].

It has been shown that exposure to sublethal ROS concentrations can stimulate the manifestation of defense reactions. This process is called hormesis [77]. Hormesis induces defense mechanisms on two levels. The first level is enzymatic (short-term reaction). At this level, antioxidant enzymes are activated. The second level is long-term adaptation. Long-term adaptation consists of two sublevels: transcriptional and genomic. At the level of transcription, ROS induces adaptation due to the activation of antioxidant mechanisms within a few hours or days [78]. At the genomic level, ROS can cause damage to the DNA structure, which activates the mechanisms for repairing DNA damage. These mechanisms include homologous recombination and excisional repair. In these mechanisms, two of the DNA polymerases responsible for DNA synthesis have poor validation activity and may include abnormal bases in DNA strands, which leads to a high frequency of spontaneous mutations and genome plasticity under adverse influences [79]. Such plasticity of the genome can lead to the development of resistance to metals and metal oxide nanoparticles [80].

The adaptation mechanisms of bacteria in relation to nanoparticles also include overexpression of extracellular substances by bacterial cells, such as flagellin, which form an extracellular matrix that promotes agglomeration and deactivation of nanoparticles [81]. Despite the existing mechanisms of adaptation of bacteria to the impact, numerous studies have noted the high antibacterial potential of ZnO nanoparticles.

LITERATURE REVIEW

Despite the apparent wide range of strains, against which nanoparticles exert the antimicrobial activity, their effectiveness against particular strains can be significantly different. As a rule, gram-negative bacteria are less sensitive to ZnO nanoparticles than gram-positive bacteria [62, 66, 82]. Somewhat higher resistance of gram-negative bacteria can be

TABLE 1 | Main characteristics, physicochemical and biological parameters of ZnO nanoparticles presented in the review.

n	Size, nm	Structure	Shape	Composition of material	Type of microorganism	BE	Concentration	Exposure time and temperature	Medium	Synthesis path	Authors
1	3	Oxo-alkoxy phase variety	Sph	The metal oxide core is surrounded by a shell of surface groups, (-OR, -OH) ZnO	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>St. aureus</i>	BS	0.5–1.5 M	24 h, 35°C	TCS	Sol-gel method	[52]
2	<50 ± 9	Cryst	Sph	ZnO	<i>V. cholerae</i> , <i>E. coli</i> , <i>C. Jejuni</i> , <i>St. aureus</i> (MRSA)	BC	0.78; 1.56; 3.125; 6.25; 12.5; 25; 50 mM	48 h, 37°C (for <i>C. Jejuni</i> 42°C)	LB medium, MHA	Chemical method followed by mechanical and heat treatment	[87]
3	50–70	Cryst	Sph	ZnO, chitosan	<i>St. aureus</i> , <i>E. coli</i>	BS	30 µL/ml	37°C	TSA	Microwave heating using chitosan as a stabilizing agent	[66]
4	18	Cryst	Sph	ZnO	<i>E. coli</i> , <i>St. aureus</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i>	BS	10–100 µg/ml	24 h, 35 ± 2 °C	NA	Sol-gel method by post heat treatment	[43]
5	9.3 ± 3.9	Cryst	Sph	ZnO	<i>M. tuberculosis</i>	BS, BC	1–64 µg/ml	24 h, 37 °C	LJ medium	Chemical deposition method	[85]
6	5.3, 33.9, 4.5, 21.2, 6.8, 38.2, 5.3, 6.7	ZnO coated with 3-glycidyoxypropyl trimethoxysilane (GPTMS) as a surface modifier	Sph	Colloidal suspensions of ZnO NPs and GPTMS-ZnO dispersions in water	<i>St. aureus</i> , <i>E. coli</i>	BS, BC	1.25–0.01 mg/ml	24 h, 37 °C	MHM	Sol-gel method with varying reaction times for NP size control followed by the addition of (3-glycidyoxypropyl) trimethoxysilane (GPTMS) as a surface modifier	[62]
7	50 (ZnO); ~1000 (GO- ZnO)	Cryst	GO- ss; ZnO- Sph	Spindle-shaped GO structures covered with crystals of ZnO NPs	<i>E. coli</i> , <i>S. typhimurium</i> , <i>B. subtilis</i> , <i>E. faecalis</i>	BC	100 µM	12 h, 37 °C	LB broth	Modified hammers method	[82]
8	~18	Cryst	Sph	ZnO	<i>St. aureus</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>P. mirabilis</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>C. tropicalis</i>	BS, BC, FS	50, 100, 200 µg/ml	24 h, 37°C for bacteria and 28°C to 35°C for yeast	MHA; for fungi Sabouraud dextrose agar	Chemical using <i>A. indica</i> water extract followed by heat treatment	[88]
9	10	Cryst	Sph	ZnO	<i>St. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i> , <i>C. albicans</i> , <i>A. niger</i> , <i>T. rubrum</i>	BC, FC	10–5187 µM	5 days-3weeks 34°C for fungi; 18 h, 34°C. For bacteria 48 h, 37°C	SDA, BHI, MH broth, SD broth	Chemical synthesis using an aqueous extract of chelidonium majus	[89]
10	33	Cryst	Sph	ZnO	<i>E. coli</i> , <i>S. choleraesuis</i> , <i>B. subtilis</i>	BS	10 mg/ml	48 h, 37°C	MHA	Chemical followed by mechanical and heat treatment	[90]
11	Length 90-100; dia 80- 90	Cryst	Rod	ZnO	<i>B. cereus</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> и <i>S. typhimurium</i>	BS, BC	0.01–100 mg/ml	24 h, 35°C	TSA	Sonochemical method	[63]
12	~30	—	Sph	ZnO	<i>C. jejuni</i> , <i>S. enterica</i> , <i>E. coli</i>	BC	0.025–0.1 mg/ml	<i>C. jejuni</i> 24 h, 42°C. <i>S. enterica</i> , <i>E. coli</i> 16 h, 37°C	MHB, LB	Used a finished commercial product, inframat advanced materials LLC (manchester, CT)	[72]
13	20	Cryst	Sph	ZnO	<i>S. typhimurium</i> , <i>S. aureus</i>	BC	1.33 mM	24 h, 37 °C	NA	Sol-gel method	[73]
14	20	Cryst	Sph	ZnO, ZnO + gelatin	<i>P. aeruginosa</i> , <i>E. faecalis</i> , <i>C. albicans</i>	BS	25; 50 µg/ml	24 h, 37 °C	NA	Chemical followed by heat treatment	[91]
15	23–26	Cryst	Sph	ZnO	<i>K. pneumonia</i> , <i>S. aureus</i> , <i>C. albicans</i> , <i>P. notatum</i>	BS	62.5–100 µg/ml	-	MHA	Chemical using powder extract of dry ginger rhizome (<i>Zingiber officinale</i>) followed by thermal and mechanical treatment	[74]
16	23.7–88.8	Cryst	Flow	ZnO	<i>S. aureus</i> , <i>E. coli</i> , <i>C. albicans</i>	BS	0.25; 0.5 mg/ml	24 h, 37°C; (<i>C. albicans</i> at 28°C)	NA, SDA	Sol-gel method at various temperatures (25°C, 35°C, 55°C, 75°C)	[64]
17	FZnO~1750–2250; BZnO ~36.71–51.80	Cryst	FZnO-flow; BZnO-Sph	ZnO	<i>E. coli</i>	BS	0.1- 0.4 g/ml	24 h, 37 °C	MHA	Wet chemical method	[63]

(Continued on following page)

TABLE 1 | (Continued) Main characteristics, physicochemical and biological parameters of ZnO nanoparticles presented in the review.

n	Size, nm	Structure	Shape	Composition of material	Type of microorganism	BE	Concentration	Exposure time and temperature	Medium	Synthesis path	Authors
18	60–70	Cryst	-	ZnO	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	BS	125–1028 µg/ml	18 h, 35 ± 1 °C	MHA	Chemical method using an aqueous extract of <i>Trifolium pratense</i> flowers, followed by heat treatment	[92]
19	ZnO ~21.05; Ag-ZnO ~30.13	Cryst ZnO, and LF ZnO, doped Ag	Sph	ZnO, ag, AgO	<i>E. coli</i> , <i>K. pneumonia</i> , <i>P. aeruginosa</i> , <i>S. typhi</i> , <i>S. aureus</i> , <i>Fusarium</i> spp., <i>R. necatrix</i>	BS, FS	100 mg/ml	8–24 h, 37 °C	NA, PDA	Chemical method using an aqueous extract of cannabis sativa leaves	[93]
20	22	Cryst	Sph	ZnO	<i>B. subtilis</i> , <i>S. mutans</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. oxytoca</i>	BS	0.0005 g (0.5 mg) weighed portions of NPs were placed in a well in agar seeded with bacteria	24 h, 37 °C	MHA	Sol-gel method	[94]
21	20–25	Cryst	Sph, hexag	ZnO	<i>S. aureus</i> , <i>S. typhimurium</i> , <i>A. flavus</i> , <i>A. fumigatus</i>	BS	20–100 µg/ml	24 h, 37 °C	MHB, LB broth	Chemical deposition method followed by thermal and mechanical treatment	[95]
22	200–500	Cryst	Sph. (ZnO), Rod (ZnO-Cu)	ZnO; ZnO, led. Mn, Fe, Co, Ni, Cu. Dopant content 3%	<i>E. coli</i>	BS	0.5 mg/ml	48 h, 37 °C	NB	Solvothermal method	[96]
23	42–64	Cryst	Sph., oval	ZnO	<i>A. hydrophila</i> , <i>E. coli</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. faecalis</i> , <i>S. pyogenes</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>C. albicans</i>	BS	1.2–25 µg/ml	24 h, 37 °C	MHA	Biosynthesis of zinc oxide nanoparticles using reproducible bacteria aeromonas hydrophila as an environmentally friendly reducing agent	[97]
24	20–50	Cryst	Sph	Chemically pure	<i>S. paratyphi</i> , <i>E. coli</i> , <i>S. aureus</i>	BS	20 µL of extract was applied to a 6 mm disc	24 h, 37 °C	MHA	Chemical using an aqueous extract of <i>Tabernaemontana divaricata</i> leaves followed by heat treatment	[98]
25	800–3000	Cryst	Flow	ZnO	<i>S. aureus</i> , <i>E. coli</i>	BS	25–125 mg/L	18–24 h, 37 °C	MHA	Chemical precipitation	[99]
26	5	Cryst	Sph	ZnO-NP nanoparticles in the form of a powder bound in a polystyrene film (ZnO-PS) or suspended in a polyvinylpyrrolidone (ZnO-PVP) gel	<i>L. monocytogenes</i> , <i>S. enteritidis</i> , <i>E. coli</i>	BS	0.1–0.5 mg/ml	48 h, 22 °C	LEW, BHI, TSB	Sol-gel method followed by heat treatment	[100]
27	4.45 ± 0.37	Cryst	Sph	ZnO	<i>S. aureus</i> , <i>E. coli</i>	BS	0.375–1.5 mg/ml for <i>E. coli</i> ; 0.09–0.375 mg/ml for <i>S. aureus</i>	24 h, 37 °C	TSB	Chemical precipitation	[101]
28	3	Cryst	-	-	<i>S. aureus</i> , <i>E. coli</i>	BS	0.5–16 mg/ml	24 h, 37 °C	NB	Sonochemical method	[102]
29	~249	Cryst	Without shape	ZnO	<i>E. coli</i>	BS	0.1.0.25 g/L	8–16 h, 37 °C	LB	Used a finished commercial product from Nanophase technologies and Nanostructured and amorphous materials	[103]
30	~20	Cryst	Sph	Zno, Ag	<i>E. coli</i> , <i>S. aureus</i>	BS	20–70 µg/ml	24 h, 37 °C	NA, LB broth	Chemical using thymus vulgaris leaf extract	[104]
31	~124.6	Cryst	Sph	ZnO	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>S. paratyphi</i>	BS	20–100 µg/ml	24 h, 37 °C	MHA	Chemical using an aqueous extract of <i>Tectona Grandis</i> (L.)	[105]

(Continued on following page)

TABLE 1 | (Continued) Main characteristics, physicochemical and biological parameters of ZnO nanoparticles presented in the review.

n	Size, nm	Structure	Shape	Composition of material	Type of microorganism	BE	Concentration	Exposure time and temperature	Medium	Synthesis path	Authors
32	ZnO 243; ZnO-Fe 197	Cryst	Rod	ZnO, Fe	<i>E. coli</i> , <i>B. safensis</i>	BC	15–25 μL	24 h, 37°C	MHA	Chemical synthesis using <i>Amaranthus spinosus</i> leaf extract as a reducing agent	[106]
33	20–30	Cryst	Rod, flow, Sph	ZnO	<i>E. coli</i> , <i>S. aureus</i>	BS	0.625–10 mg/ml	24 h, 37 °C	NB	Solvothermal method	[107]
34	4, 10, 30	Cryst	Sph	ZnO	<i>E. coli</i> , <i>S. aureus</i>	BS	12.5–1000 $\mu\text{g/ml}$	24 h, 37 °C	LB broth, NA	Solvothermal method	[108]
35	70	Cryst	Sph, rod	ZnO	<i>E. coli</i>	BS	0, 3, 6, 12 mM/L^{-1}	12 h, 37 °C	TSA	Used ready-made commercial material from alfa aesar (ward hill, MA, USA)	[68]
36	ZnO 13.79; ZnO-Mn (5% Mn) 16.72; ZnO-Mn (10%Mn) 17.43	Cryst	Seed-like	ZnO, Mn	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>S. dysenteriae</i> , <i>S. typhi</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , <i>S. aureus</i>	BS	50–250 $\mu\text{g/ml}$	24 h, 37 °C	MHA	Chemical deposition method	[109]
37	3–25	Cryst	Spiny Hexag	ZnO	<i>B. subtilis</i> , <i>E. coli</i> , <i>C. albicans</i>	BS	0.5 mg/ml	24 h, 37 °C	NA	Sol-gel method	[110]
38	<5	Cryst	Hexag (CE-ZnO-NP); triang (WP-E-ZnO-NP)	ZnO	<i>S. epidermidis</i> , <i>B. subtilis</i> , <i>K. pneumoniae</i> , <i>P. aeruginosa</i>	BS	10 $\mu\text{g/ml}$	24 h, 37 °C	NA	Biogenic synthesis of NP-ZnO using water callus extract (CE) and whole plant isodon rugosus obtained <i>in vitro</i> (WP-E)	[111]
39	~28	Cryst	Sph	ZnO	<i>K. aerogenes</i> , <i>E. coli</i> , <i>P. desmolyticum</i> , <i>S. aureus</i>	BS	200,400 $\mu\text{g/well}$	36 h, 37 °C	NA	Biogenic synthesis using an aqueous extract of <i>Ruta graveolens</i> stems as a reducing agent	[112]
40	66	Cryst	Hexag	ZnO	<i>S. aureus</i> , <i>Proteus</i> sp., <i>Acinetobacter</i> sp., <i>P. aerogenes</i> , <i>E. coli</i>	BS	20 $\mu\text{g/ml}$	24 h, 37 °C	NA	Synthesis using <i>Ficus carica</i> leaf extract	[113]
41	14.18	Cryst	Sph	ZnO	<i>M. luteus</i> , <i>S. aureus</i> , <i>S. pneumoniae</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	BS	70–150 $\mu\text{g/ml}$	24 h, 37 °C	MHA	Synthesis using <i>Rubia Cordifolia</i> root extract	[114]
42	~1000	Cryst	Flow	Ag and TiO ₂ nanoparticles on the surface of large ZnO particles in the shape of a flower	<i>E. coli</i>	BS	0.4 g/L	24 h., 37 °C	LB	Simple hydrothermal synthesis	[115]
43	164 ± 2	Cryst	Sph	Sodium alginate (SA)/poly (vinyl alcohol) (PVA) fiber mats containing ZnO NPs	<i>S. aureus</i> , <i>E. coli</i>	BS	SA/PVA with various concentrations of ZnO nanoparticles (0.5, 1, 2 and 5%)	24 h, 37 °C	LB broth, NA	Sol-gel method	[116]
44	20.99-32.24	Cryst	Hexag	ZnO, ZnO + Fe (1–17%)	<i>E. coli</i> , <i>P. aeruginosa</i>	BS	1–8 mg/ml	24 h, 30 °C	NA	Sol-gel method	[117]
45	2–28	Cryst	Sph	ZnO	<i>Pseudomonas</i> sp., <i>Fusarium</i> sp	BS, FS	10 ⁻¹ M	24 h, 37 °C	NA	Chemical method including the use of surfactants under various conditions	[118]
46	60–80	Cryst	Sph	NP-ZnO, evenly distributed in the polymer medium	<i>E. coli</i> , <i>S. aureus</i>	BS	—	24 h, 37 °C	-	One-stage plasma synthesis of thin nanocomposite films of polymer/NP-ZnO by co-precipitation of a renewable polymer based on geranium essential oil and zinc nanoparticles obtained as a result of thermal decomposition of zinc acetylacetonate	[119]
47	>100	Cryst	Sph	ZnO, ZnO coated with TG (thioglycerol)	<i>E. coli</i>	BS, BC	8–55 mg/100 ml	12 h, 37°C	LB	Wet chemical synthesis (Continued on following page)	[71]

TABLE 1 | (Continued) Main characteristics, physicochemical and biological parameters of ZnO nanoparticles presented in the review.

n	Size, nm	Structure	Shape	Composition of material	Type of microorganism	BE	Concentration	Exposure time and temperature	Medium	Synthesis path	Authors
48	Dia 800 ± 200	Cryst	Spiny	ZnO-NP with surface modification (annealing in an Ar medium at a temperature below 800°C, with the removal of Zn and an increase in O vacancies; plasma oxidation at temperatures above 1000°C)	<i>E. coli</i> , <i>S. aureus</i>	BS	8 mg/ml	24 h, 37°C	NA	Vapor-liquid-solid (VLS) method with subsequent surface modification by annealing in an argon atmosphere with a deficiency of O ₂ and oxidation during plasma treatment	[106]
49	40–1200	Cryst	Sph., rod	ZnO, ZnO + PEG (polyethylene glycol), ZnO + starch	<i>E. coli</i> , <i>S. aureus</i>	BS	1 μM - 7 mM	24 h, 37°C	LB	Wet chemical synthesis	[107]
50	10–60	Cryst	Sph	ZnO	<i>A. baumannii</i>	BC	1–50 mM	12 h, 37°C	LB broth	Sol-gel method using an aqueous extract of <i>Calotropis procera</i> leaves	[86]

BE - Biological effect. BC-bactericidal effect. BS- bacteriostatic effect. FS- fungistatic effect. Cryst-crystalline. Rod-rod-shaped. Sph-spherical. Triang-triangular. Flow-flower-shaped. Hexag-hexagonal. Spiny-spiny-shaped.

explained by the peculiarities of their cell wall structure. In contrast to gram-positive bacteria, the cell wall of gram-negative bacteria includes the additional outer membrane containing lipopolysaccharides (LPS) [83]. It is shown that LPS can improve the barrier properties of the outer membrane and, therefore, increase bacterial resistance, in particular, to antibiotics [84]. Epidemiologically significant microorganisms deserve a special attention, for example, *Mycobacterium tuberculosis*, against which ZnO nanoparticles exert the bacteriostatic effect but not bactericidal [85].

On the contrary, several microorganisms (for instance, *Campylobacter jejuni*) have an increased sensitivity to ZnO nanoparticles, which make them a convenient model for studying molecular mechanisms of the antimicrobial effect of nanoparticles [24]. ZnO nanoparticles (ZnONPs) disturb the processes of bacterial DNA amplification, reduce expression of a wide range of genes of *C. jejuni* that are responsible for virulence, significantly alter expression of genes of oxidative and general stress [24]. An important feature of ZnO nanoparticles used in one of the studies is the antibacterial activity against resistant bacterial strains, for example, carbapenem-resistant *Acinetobacter baumannii* (RS-307 and RS-6694) [86]. The dependence of effectiveness on a bacterial growth phase was shown for ZnO nanoparticles. In particular, ZnO nanoparticles are effective against gram-negative and gram-positive bacteria at the exponential growth phase; however, the antibacterial properties of nanoparticles are significantly decreased at the lag and stationary phases [52]. A range of bactericidal concentrations of ZnO nanoparticles is usually significantly less than a range of 4 [62]. At present, an active search for methods to increase the antimicrobial action of nanoparticles is carried out. Below we present the literature search. Nanoparticles are classified by the method for synthesis, size, structure, form, absence or presence of the envelope

or nucleus. The objects, on which nanoparticles influenced, are classified by types, biological effect of nanoparticles, concentration of nanoparticles, duration of exposure, temperature and environment. The data are presented in **table 1**.

Let us consider proposed methods for increasing antibacterial properties of ZnO nanoparticles. The first method for increasing antibacterial properties of ZnO nanoparticles is to use a combination of different metal compounds [52, 90]. For example, the CuO and ZnO have comparable effectiveness against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* at the exponential growth phase. ZnO nanoparticles were practically inactive at the lag and stationary phases, while CuO nanoparticles retained the significant activity [52]. Ag and ZnO nanoparticles in different ratios inhibit the growth of antibiotic resistant *Mycobacterium tuberculosis* strains but did not lead to bacterial death [85]. ZrO₂-ZnO nanoparticles have the pronounced antimicrobial action in contrast to ZrO₂ nanoparticles, but the antimicrobial effect of ZrO₂-ZnO nanoparticles does not exceed that of ZnO nanoparticles [94]. However, combinations of metal oxides not always give the synergetic effect. In particular, CdO-ZnO nanoparticles have the antimicrobial action comparable with that of CdO nanoparticles [90]. Doping of ZnO nanoparticles with the Fe ions enables achieving a significant antibacterial effect against *E. coli*, *Pseudomonas aeruginosa* [117]. TiO₂/ZnO nanoparticles have more pronounced bactericidal effect against *E. coli* compared to ZnO nanoparticles. Ag/TiO₂/ZnO nanoparticles are more effective than TiO₂/ZnO nanoparticles [115]. Compared to ZnO nanoparticles, ZnO-Mn nanoparticles have higher antimicrobial activity against *K. pneumoniae*, *Shigella dysenteriae*, *S. enterica Typhimurium*, *P. aeruginosa* and other bacteria [109].

The second method for increasing antimicrobial effectiveness is to use combinations of ZnO nanoparticles and carbon

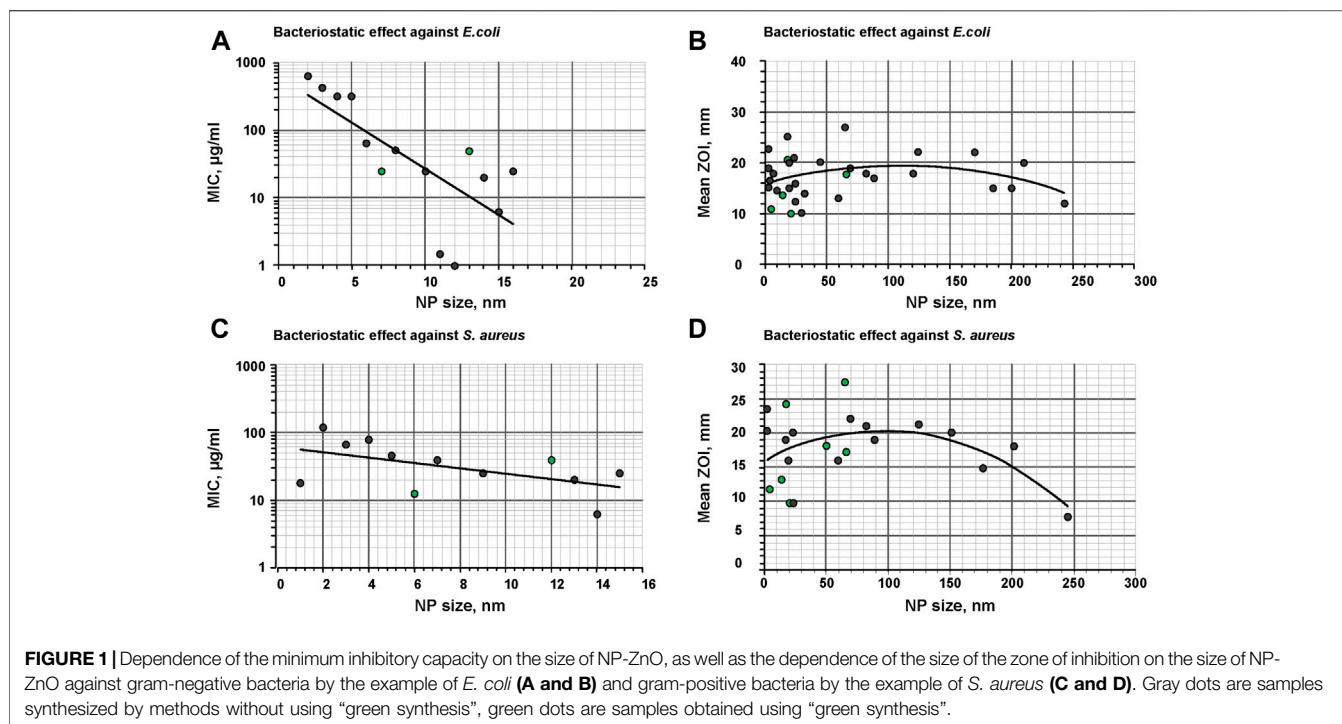


FIGURE 1 | Dependence of the minimum inhibitory capacity on the size of NP-ZnO, as well as the dependence of the size of the zone of inhibition on the size of NP-ZnO against gram-negative bacteria by the example of *E. coli* (A and B) and gram-positive bacteria by the example of *S. aureus* (C and D). Gray dots are samples synthesized by methods without using “green synthesis”, green dots are samples obtained using “green synthesis”.

nanoparticles, in particular, spindle-shaped graphene oxide (GO) nanoparticles [68, 108, 109]. It is shown that GO-ZnO nanoparticles effectively inhibit the growth of gram-negative (*E. coli*, *S. typhimurium*) and gram-positive (*Bacillus subtilis*, *Enterococcus faecalis*) bacteria [68]. With that, the antibacterial effectiveness of the mixture of GO-ZnO nanoparticles turned to be nearly twice as high as that of ZnO nanoparticles and almost four times higher than that of GO nanoparticles [82].

The third method is coating ZnO nanoparticles with modifying agents. Gelatin-coated ZnO nanoparticles showed higher inhibition of the growth of gram-negative bacteria compared to gram-positive bacteria [91]. As was mentioned above, overcoming antibiotic resistance in gram-negative bacteria is a more difficult task. Gelatin-coated ZnO nanoparticles inhibit biofilm formation of *C. albicans* (an additional resistance factor) [91]. These nanoparticles also inhibit angiogenesis in chick embryos, which makes them candidates for the development of preparations preventing undesirable angiogenesis [91]. The chemical surface modification of nanoparticles using (3-glycidyloxypropyl) trimethoxysilane (GPTMS) and decrease in a size up to 5 nm lead to an increase in antimicrobial effectiveness of nanoparticles against *S. aureus* [62]. Treatment with polystyrene increased the bacteriostatic effect of ZnO nanoparticles against *E. coli* and *Listeria monocytogenes*; with that, uncoated ZnO nanoparticles did not have the bacteriostatic effect against *L. monocytogenes* [100]. Modification of ZnO nanoparticles with polyethylene glycol or starch also alters properties of nanoparticles [121]. Modification with polyethylene glycol increased the bacteriostatic effect of ZnO nanoparticles against *E. coli* и *S. aureus*; with that, effectiveness against gram-negative bacteria

was higher. Polyethylene enhanced cytotoxicity of ZnO nanoparticles toward the cancer cell line (MG-63) by induction of apoptosis. Modification with starch allowed retention of antibacterial properties of ZnO nanoparticles and reduction of cytotoxicity compared to modification with polyethylene glycol [121]. Treatment with thioglycerol, contrary to the expectations, did not increase the bacteriostatic and bactericidal activity of ZnO nanoparticles [71]. Polymer films from sodium alginate/polyvinyl alcohol gained bacteriostatic properties after incorporation of ZnO nanoparticles, which can be used in the development of more durable materials [116].

The fourth method is modification of the synthesis method leading to changes in the geometrical characteristics of nanoparticles. ZnO nanoparticles synthesized by the sonochemical method have more pronounced inhibitory properties against *Bacillus cereus*, *S. aureus*, *S. Typhimurium* and *Pseudomonas aeruginosa* than ZnO nanoparticles synthesized by the classical physio-chemical methods [63]. Nanoparticles synthesized at comparatively low temperatures are flower-shaped and have the comparable antimicrobial activity against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria and, to a lesser extent, fungi (*C. albicans*) [64]. When using ROS photocatalytic generation and release of Zn^{2+} , flower-shaped ZnO nanoparticles show more pronounced antimicrobial activity against *E. coli* than more lacunary hexagon-shaped ZnO-NPs [70].

Antibacterial properties of nanoparticles depend on their size [122-124]. For several nanoparticles, the highest antibacterial activity is achieved at the smallest size [103, 107, 125]; however, we have not found in the literature a clear dependence of antibacterial effectiveness on a nanoparticle size. We had to

analyze literature by ourselves and build a graph reflecting a dependence of an inhibition zone size on a size of ZnO nanoparticles (**Figure 1**). Analysis of literature allows stating that the highest potential antimicrobial effectiveness of nanoparticles against both *E. coli*, and *S. aureus* is observed at a nanoparticle size of about 100 nm. It is necessary to note that “green chemistry” not always leads to synthesis of effective nanoparticles. For example, in studies on *S. aureus*, only two types of nanoparticles out of six (33%) had the antibacterial activity at a level higher than average. When studying on *E. coli*, only one of five (20%) types of nanoparticles generated using “green chemistry” exerted the antibacterial activity at a level higher than average. Therefore, it can be suggested that nanoparticles generated by “green chemistry” still have insufficient effectiveness.

As can be seen in **Figures 1B,D**, quite high dispersion of effectiveness is seen in the region of small sizes of nanoparticles (1–50 nm). Therefore, we studied the dependence of the minimum inhibitory concentration (MIC) on sizes of ZnO nanoparticles (**Figures 1A,C**). It is shown that the use of nanoparticles with sizes of up to 10 nm is not effective. Usually, at these average sizes of nanoparticles, distribution of nanoparticles by sizes is rather complex and not always narrow. Nanoparticles with small sizes are quite prone to aggregation. Apparently, high dispersion of antibacterial activity at small sizes of nanoparticles can be explained by this fact.

Flower-shaped ZnO nanoparticles can reach large sizes (up to 3 μm) and demonstrate the antimicrobial activity against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria [99]. For spherical ZnO nanoparticles, the antimicrobial activity practically does not depend on the type of a targeted organism. Hexagonal ZnO nanoparticles have higher bactericidal activity against antibiotic resistant *Staphylococcus epidermidis*, *B. subtilis*, *Klebsiella pneumoniae* and *P. aeruginosa* strains compared to ZnO-NPs with the triangular shape [111]. Thorn-like ZnO nanoparticles cause significant reduction in the growth of *B. subtilis*, *E. coli* and *C. albicans* colonies demonstrating the antibacterial and antifungal activities [110].

The fifth method is modification by physio-chemical methods, for example, by annealing in the Ar environment at high temperatures, or plasma oxidation. With that, the effects of modification can be different: Ar annealing decreases the antibacterial activity of ZnO nanoparticles, while plasma oxidation improves antibacterial properties of ZnO nanoparticles against *E. coli* and *S. aureus* [120]. The sixth method is the use of additives causing photocatalysis of reactive oxygen species (ROS). This modification enables a significant increase in antibacterial properties of ZnO nanoparticles [70, 93]. The seventh method is the so-called “green synthesis” [126–128]. ZnO nanoparticles generated by “green synthesis” have the antimicrobial activity against gram-negative and gram-positive bacteria, as well as several fungi of the genus *Candida* [88]. In turn, nanoparticles synthesized using the *Tabernaemontana divaricata* extract demonstrated the

antibacterial activity against *S. aureus*, *E. coli* and lower activity against *S. enterica* Paratyphoid [98]. The eighth method is a change in the environment conditions. At acidic pH levels, ZnO nanoparticles had higher bacteriostatic action against *S. aureus* and *E. coli* than at neutral pH [101]. The combination of all approaches described above can be most promising, for example, the use of Ag-ZnO nanoparticles synthesized in the *Cannabis sativa* extract. The generated nanoparticles can be used in combination with photocatalysis and have the antibacterial and antifungal activities.

CONCLUSION

Zinc oxide nanoparticles have significant antibacterial potential. The use of various methods of synthesis, chemical modification, as well as joint use with other nanomaterials affects the physical and morphological characteristics of nanoparticles, which, in turn, leads to a change in their antibacterial properties. As a result, nanoparticles based on zinc oxide are increasingly used not only in nanoelectronics and optics, but also in such industrial areas as cosmetic, food, rubber, pharmaceutical, household chemicals, etc. The use of packaging with incorporated zinc oxide nanoparticles is possible will allow in the future to prevent the growth of microorganisms and spoilage of food. In turn, the use of medical dressing materials containing ZnO nanoparticles will allow avoiding microbial contamination of the wound and promotes its early healing. Thus, zinc oxide nanoparticles can be considered as a promising new generation antimicrobial agent.

AUTHOR CONTRIBUTIONS

SG designed this topic. DB, MR contributed to collecting related references. DB made a table. DS, SG, MR wrote most of the manuscript. AS and AL were involved in discussing the manuscript and translating it into English.

FUNDING

This work was supported by the Ministry of Science and Education of the Russian Federation (Grant Agreement 075-15-2020-775).

ACKNOWLEDGMENTS

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references to this manuscript. The authors are also grateful to authors/editors/publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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