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Review Article

# Nanobiotechnology against *Salmonella spp*

Dragomira S. Stoyanova[1]*, Iliana A. Ivanova[1], and Todorka G. Vladkova[2]

[1]*Department of General and Industrial Microbiology, Sofia University, Bulgaria*
[2]*Polymer department, University of Chemical Technology and Metallurgy, Bulgaria*

*Corresponding author
Stoyanova D, Department of General and Industrial Microbiology, Sofia University, Bulgaria, Tel: 359894633923; Email: dragomira.stoyanova@abv.bg

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**Abstract**

This article aims to summarize the benefits of nanobiotechnology in the fight against socially and economically significant bacterial species like *Salmonella*, starting from identification and diagnostics, drug delivery, increasing the efficiency of conventional antibiotics, just like and antibacterial ability of nanoparticles in their alone application. A special attention on the method of synthesis and trend of green technologies was paid. In an attempt to draw conclusions and dependencies, a systematic review on the mechanisms of action of various nanoparticles against *Salmonella spp*., clarified until nowadays is prepared.

## ABBREVIATIONS

NP(s): Nanoparticle(s); MIC: Minimal Inhibition Concentration; MBC: Minimal Bactericidal Concentration

## INTRODUCTION

*Salmonella spp*. is delicate, non-spore forming gram negative rod-shaped bacteria of the family *Enterobacteriaceae,* which are widespread in the environment. Intestinal tract of many animals and humans is their usual place of habitat and propagation, hence they are released into the environment and can survive for a long time and grow in food, plant and animal waste, in general, wherever they have found organic matter and suitable conditions [1].

*Salmonella* is found to be infecting 21.7 million people and literally causing 2,17,000 deaths annually [2]. The main route of transmission for enteric fever is the polluted water and food [3,4].Other study proved that *S. typhi* is frequently associated with the gallstones in asymptomatic human carriers, in which the bacteria colonizes and forms biofilm [5]. The appearance of severe infections, caused by *Salmonella spp*. due to its ability of biofilm formation in food manufacturing and processing plants is reported in [6]. These pathogens cause enormous losses in the food industry [6].

## IDENTIFICATION AND DIAGNOSTIC

Taxonomic differentiation of the genus *S. enterica spp*. is complicate and time consuming because of its resemblance with *E. coli* and about 2,400 serovars. Different fast test were created, but the use of nanoparticles such as gold has further improved biosensor sensitivity and provides a fast detection through direct visualization [7].The use of DNA and gold nanoparticles (Au NP) simultaneously have led to the development of a class of colorimetric biosensors in which detection results are readily observed from the test kit rather than to use further equipment for visualization purposes [8,9]. This in turn greatly reduces the overall cost for the detection of *S. enterica* [10].

Thavanathan et al. [10], developed a colorimetric biosensor using a dual platform of gold nanoparticles and grapheme oxide sheets for the detection of *Salmonella enterica*. In this original research the presence of the invA gene in *S. enterica* causes a change in color of the biosensor from its original pinkish-red to a light purplish solution. This occurs through the aggregation of the primary gold nanoparticles–conjugated DNA probe onto the surface of the secondary graphene oxide– conjugated DNA probe through DNA hybridization with the targeted DNA sequence. The novelty of this biosensor design is that the unmodified targeted gene sequence acquired from spike food samples acts as a cross-linker between the Au NP and GO probes, which induces an observable color change when both nanoparticles are brought together [10]. The dual nanoparticle platforms of Au NP and GO function as the color change and binding components. Through its surface plasmon resonance properties of Au NP, the color of the biosensor could be manipulated by controlling the distance between nanoparticles in the event of DNA hybridization [11]. GO through its numerous surface modifications and transparent nature provides the ideal platform for the aggregation and binding of the Au NP [12].

Thavanathan et al., determined the specificity of the biosensor by measurement and characterization in two parts. The biosensor was tested with the nine most common serovars found in contaminated food. The invA gene targets from *S. typhi, S. typhimurium, S. enteritis, S. paratyphi A, S. covallis, S. heidelberg, S. stanley, S. weltevreden, and S. choleraesuis* were

obtained through PCR amplification. Spectrophotometry analysis of all nine biosensor samples showed a wavelength shift from an initial 525 nm to approximately 600 nm, which indicated the aggregation of the Au NP due to hybridization between the biosensor DNA probes and the target DNA. Minor wavelength peak at 570 nm have showed almost all the *S. enterica* serovars. These appearances of multiple wavelength peaks could be attributed to the varying sizes of the GO-DNA sheets present within the biosensor solution. This causes the diversity in aggregation of the Au NP-DNA, which is spread throughout the entire biosensor solution and not localized onto a single point, thus giving out multiple wavelength peaks rather than a single peak with the spectrophotometry analysis [10]. Furthermore, the authors of this study determined the limit of detection for the biosensor through the use of multiple concentrations of complementary gene target. Analysis was done through a dual confirmation process, which involved a shift in wavelength peak in spectrophotometry analysis and observable color change of the biosensor solution from pinkish-red to light purplish. The limit of detection for the biosensor through its final color change was found to be at 0.98 nM of DNA target, while with the addition of 0.49 nM of the gene target, the biosensor solution remained pinkish-red without any change [10].

Growing interest in the aptamer-based biosensors is reported. Their advantages are discussed in detail in the review of V. Gedi and Y. Kim [13].

As single-stranded nucleic acids (ssDNA or RNA), aptamers offer several advantages over other sensing molecules for diagnostic and therapeutic applications [14]. In addition to being chemically stable, cost-effective and producible on a large scale, aptamers also possess an intermediate size (between antibodies and small peptides) and have comparable or higher binding affinities for their targets. These targets range from small molecules to whole cells [15-17] via an *in vitro* selection process known as systematic evolution of ligands by exponential enrichment (SELEX) [18].

Unlike antibodies based on purified receptors, aptamers are more attainable and responsive in living cells because they are selected from intact cells; it is not necessary to consider the conformational changes of the recognition domains in the cell membrane. Among many other types of cells, bacteria and tumor cells have been of primary interest [19,20], due to their involvement in many human diseases. To exploit the full potential of aptamer-based cell targeting, aptamers can be combined with nanomaterials, such as gold nanoparticles (AuNPs), silica NPs (SiNP), graphenes, magnetic NPs (MNP) and quantum dots (QDs). This aptamer-nanomaterial hybridization process is easily accomplished due to the simple chemical modification and well-defined structures of aptamers [21,22]. These hybrid materials are expected to improve target diagnosis and therapy with higher sensitivity and selectivity compared to aptamer-only-based targeting strategies. Most importantly, due to the large surface area, multivalent structure, and relevant physiochemical properties of nanomaterials, aptamer-nanomaterial hybrids should provide signal amplification and an increased target binding affinity in a multivalent manner.

Wu et al. also demonstrated the salt-induced color change of AuNPs for the detection of *E. coli* and *Salmonella typhimurium* [23]. They incubated Apt-AuNPs with the target bacterial cells for 10 min. These were then aggregated upon the addition of NaCl. The subsequent color change from red to purple was simply detected either visually or with UV-vis spectroscopy [24]. This colorimetric method was able to detect $10^5$ CFU.mL$^{-1}$of *E. coli* or *S. typhimurium* [23] without requiring any expensive instrumentation or labeling process. Likewise, bacterial cells were measurable on the GO surface using a FAM (carboxyfluorescein)-aptamer which was specific for *S. typhimurium*. The increased fluorescence in the presence of the target bacteria was quantified as a function of bacterial cells, and the aptamer-based sensor reached an LOD as low as 100 CFU.·mL$^{-1}$ [25].

This review shows that by utilizing cell-SELEX, recent advances in the development of aptamer-nano hybrid sensors have led to remarkable improvement in targeting cell. Since aptamers can be easily generated and modified with various nanomaterials, the traditional limits, related to low sensitivity, poor stability, and high cost can be overcome. Aptamer-nanomaterial hybrids show tremendous potential as robust diagnostic and therapeutic reagents for detecting and characterizing different types of cells [13].

## DRUG DELIVERY

A. Nokhodchi et al., in their review article [26] clarify the challenges in drug delivery to combat *Salmonella spp*.

Despite the discovery of new antibiotics, treatment of intracellular infections often fails to eradicate the pathogens completely. One major reason is that many antimicrobials are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibitory or bactericidal effects on the intracellular bacteria [27]. In addition, antimicrobial toxicity to healthy tissues poses a significant limitation to their use [27]. Therefore, the delivery of the drug to the bacterial cells is currently a big challenge to the clinicians. This is on top of the problems posed by the emerging Multi-Drug Resistant species. Moreover, the reduced membrane permeability of microorganisms has been cited as a key mechanism of resistance to antibiotics [28].

One of the distinguishing features of liposomes is their lipid bilayer structure, which mimics cell membranes and can readily fuse with the cell membrane and deliver the antibiotic contents into the cellular cytoplasm. As a result, drug delivery may be improved to bacterial and eukaryotic cells alike. By direct fusing with bacterial membranes, the drug pay loads of liposomes can be released into the cell membranes or to the interior of the bacteria. In terms of extracellular pathogens, improved antibiotic delivery into the bacterial cells is of particular importance especially since it can interfere with some of the bacterial drug-resistance mechanisms which involve low permeability of the outer membrane or efflux systems [29].

Liposomes are particularly successful in eradicating intracellular pathogens [30]. Liposomal chemotherapeutics for the treatment of salmonellosis may employ some of the conventional antibiotics with proven inhibitory or bactericidal effect *in vitro*. Bacterial gastrointestinal infections with *Salmonella typhi* may be treated with chloramphenicol.

Alternatives to chloramphenicol include amoxicillin, co-trimoxazole and trimethoprim [31]. Recently treatment with cephalosporins and fluoroquinolones has become popular, as several members of these antibiotic families have been shown to be effective. The treatment of paratyphoid fever is the same as that for typhoid [31]. *Salmonella* food-poisoning is self-limiting and does not require antibiotic therapy, unless the patient is severly ill or blood cultures indicate systemic infection. In this case, third generation cephalosporins or fluoroquinolones are the most reliable agents [31]. Ceftriaxone or a first generation fluoroquinolone such as ciprofloxacin, ofloxacin or pefloxacin but not norfloxacin have been recommended as the first choice in typhoid and paratyphoid by The Sanford Guide to Antimicrobial Therapy [32]. The improved efficiency of liposome formulations of antibiotics has been shown *in vitro* and *in vivo*. The *in vitro* infection models utilize macrophages infected with *Salmonella*.

Nanoparticles are able to adsorb and/or encapsulate a drug, thus protecting it against chemical and enzymatic degradation. Generally, the drug is dissolved, entrapped, encapsulated or attached to a NP matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Owing to their nature, nanoparticles may be more stable than liposomes in biological fluids and during storage. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed [33]. In order to minimize the side effects of nanoparticles, the polymers associated with them must be degraded *in vivo* due to intracellular polymeric overloading. Thus in recent years, biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices in view of their applications in the controlled release of drugs, their ability to target particular organs, as carriers for DNA in gene therapy, and their ability to deliver proteins, peptides and genes through a peroral route of administration [33]. The *in vitro* interaction between [3H] ampicillin-loaded poly-iso-hexyl-cyanoacrylate nanoparticles and murine macrophages infected with *Salmonella typhimurium* showed that the uptake of nanoparticle-bound [3H] ampicillin by non-infected macrophages was six- and 24-fold greater respectivelycompared to free [3H] ampicillin. Surprisingly bactericidal activity against intracellular *Salmonella typhimurium* was similar between nanoparticle-bound ampicillin and free ampicillin. This unexpected result can be explained by bacterium-induced inhibition of phagosome-lysosome fusion within the macrophages, thereby preventing contact between the bacteria in the phagosomes and the nanoparticles in the secondary lysosomes [34].

One of the problems with antibiotic loaded nanoparticles is that in some cases the capacity of a polymeric drug carrier should be engineered to incorporate high concentrations of antibiotics to achieve the required dosage, yet avoid side effects that may be associated with higher amounts of carriers. This seems a difficult task, however, Ranjan et al., introduced two novel technologies by which high concentrations of gentamicin could be incorporated into the nanocomposits [35]. Ranjan et al., made an attempt to enhance antibacterial efficacy of gentamicin using a new technology called core-shell nanostructures Nanostructures administered *in vivo* either at multiple dosage of 5 microg g(-1) or single dosage of 15 microg g(-1) in AJ-646 mice infected with *Salmonella* resulted in significant reduction of viable bacteria in the liver and spleen. Histopathological evaluation for concentration-dependent toxicity at a dosage of 15 microg g(-1) revealed mineralized deposits in 50% kidney tissues of free gentamicin-treated mice which in contrast was absent in nanostructure-treated mice. Thus, encapsulation of gentamicin in nanostructures may reduce toxicity and improve *in vivo* bacterial clearance [35].

Later, Ranjan et al. [36**]**, incorporated gentamicin into macromolecular complexes with anionic homo- and block-copolymers via cooperative electrostatic interactions between cationic drugs and anionic polymers [36].Their study showed that in addition to the high loading of drug carried by these polymeric nanoplexes, the nanoplexes can potentially improve targeting of interacellular pathogens such as *Salmonella* [36].

## Enchanced effect of antibiotics by nanoparticles

Enteric fever remains an important public health problem in many countries of the world. In recent years, an increasing number of salmonellosis outbreaks have been recorded around the world, and probably there should be more cases that should reported [37].

Typhoid fever is endemic in developing countries especially in Southeast Asia and Africa. *Salmonella* gastroenteritis is usually a self limiting disease. Fluid and electrolyte replacement may be indicated in severe cases. Because antibiotics do not seem to shorten the duration of symptoms and may actually prolong the duration of convalescent carriage, they are not routinely used to treat uncomplicated non typhoidal *Salmonella* gastroenteritis. Current recommendations are that antibiotics should be reserved for patients with severe disease or patients who are at high risk of invasive disease [38].

Presently, quinolone, macrolide and third – generation cephalosporin antibiotics are preferred for empiric therapy pending sensitivities. Unfortunately, sensitivity to quinolones has been steadily declining: some *S.enterica* serovar *typhi* strains resistant to fluoroquinolones have already been reported [28,39,40]. Mutations in regulator genes have been shown to induce the overproduction of efflux and the down-regulation of porin synthesis. In addition, various compounds such as salicylate, imipenem or chloramphenicol are able to activate the MDR response. This phenomenon has been observed both *in vitro* during culture of bacteria in the presence of drugs and *in vivo* during antibiotic treatment of infected patients. These effectors activate the expression of specific global regulators, marA, ramA, or target other genes located downstream in the regulation cascade [28].

In recent decades, there has been increasing interest in nanoparticles production from fungi, which are expected to produce high level of silver nanoparticles. Because of their rapid growth and high rate of production, fungi are used as bio-manufacturing units, which will provide an added benefit in being easy to use as compared to other microorganisms. The combination of antibiotics and metal naoparticles could increase the antibiotic efficacy against resistant pathogenes. Nanoparticle - antibiotic conjugates lower the amount of both agents in

the concentration, which reduces harmfulness and increases antimicrobial properties. These conjugates were effective against resistant bacteria species due to this conjugation the concentrations of antibiotics were increased at the place of antibiotic –microbe interaction and thus accelerate the binding microbes and antibiotics [41].

G. Krishna et al. in their work [42] used the culture filtrate to develop a simple cost effective, biocompatible and ecofriendly approach for the extracellular biological synthesis of silver nanoparticles using *Tramates* sp. The study involved the systematic analysis of the antibacterial activity of the biologically prepared silver nanoparticles against *Salmonella sp.* They also investigated the effect of combination of antibiotics with silver nanoparticles against *Salmonella sp.* It is clear that such systems may improve the antibiotic efficacy by increasing the drug concentration with the attachment of the nanoparticles in the surrounding of the bacteria [42].

It was found that the silver nanoparticles from *Tramete ssp.* enhanced the reaction rates of the antibiotics in a synergistic mode as well as in its own way on these pathogenes. In both the cases of *S.typhi* and *S.paratyphi* the highest zone of inhibition was found in the combination of ofloxacin + silver NPs followed by ceftriaxone + silver NPs, ofloxicin, ceftiaxone and silver NPs [42].

Very interesting is approach of H. M. Ahmed (2014). The aim of his work is to study the synergistic effect of antibiotic Amikacin with biogenically synthesized silver NPs (plant extract of Nepali hog plum *Choerospondia saxillari*) and chemically synthesized silver NPs, using sodium citrate [43]. Amikacin antibiotic has showed an enhanced antibacterial activity in combination with plant extract synthesized AgNPs compared to Amikacin in combination with chemically synthesized AgNPs. The antibiotic showed 9,66% fold increase against *Salmonella typhi* with chemically synthesized AgNPs. However, AgNPs with plant extract (30g/ml) showed 20,16% fold increase against *S. typhi*[43].

## Antibacterial effect of nanoparticles against *Salmonella sp.*

*Salmonellasp*. are commonly found in the environment and there are many instances throughout the grow-out phase in which birds can come into contact with *Salmonella* and other pathogens. Laboratory trial and other two separate field trials were conducted to evaluate the efficacy of various disinfectants on the isolated *Salmonella enteriditis* when applied to poultry house floors, as well as an innovative trial also, carried out to evaluate the efficacy of same disinfectants when they contained Ag nanoparticles. [44]. White wash and iodophores containing Ag nanoparticles showed highly significant ($P < 0.05$) reduction of *Salmonella* populations in floor after disinfection process (5; 4 log10 reduction, respectively). Interestingly, *Salmonella* populations completely destroyed when exposed to phenuique and formalin containing Ag nanoparticles in field trial. This may be due to the ubiquitous nature of Ag nanoparticles, which are able to enhance the disinfectant power [44].

A. Saxena et al., have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of onion (*Allium cepa*) extract. They study the antibacterial property of silver nanoparticles toward *E.coli* and *Salmonella typhimurium* [45].

Silver nanoparticles were synthesised by this method having 33.6 nm average mean size. The preparation of nanoparticles by using onion extract has desired quality with low cost and convenient methods. These nanoparticles at concentration 50µg/ml were showed complete antibacterial activity against *E.coli* and *Salmonella typhimurium* [45].

Irayyif et al., investigate the effect of silver nanoparticles on the food borne pathogens like *Salmonella typhi* and *Salmonella paratyphi*. The silver nanopartciles at 10nm size were found to be effective towards the food borne pathogens *Salmonella*. Both the species of *Salmonella (Salmonella typhi* and *Salmonella paratyphi*) showed sensitivity to the nanopartciles. The study on the antibacterial activity, biofilm formation and their primary adherence capacity all proved of the role of the nanoparticles as antimicrobial agents. Moreover, a keen study observed that the response was stronger towards *Salmonella paratyphi* than *Salmonella typhi*. The effect of nanoparticles on the biofilm formation and primary adherence assay was found to be concentration dependent [46].

Ravikumar et al., in their study investigate the antibacterial potential of metal oxide nanoparticles viz., $Al_2O_3$, $Fe_3O_4$, $CeO_2$, $ZrO_2$, and MgO against poultry pathogens viz., *Klebsiella sp*., *E. coli*, *Staphylococcus sp*. and *Salmonella sp*. The $ZrO_2$ showed maximum antibacterial activity against *Salmonella sp.* followed by *E. coli* respectively. The MIC and MBC results revealed that, the $ZrO_2$ nanoparticles inhibit the bacterial growth at a concentration of 2.5µg/ml against *Salmonella sp*. All the nanoparticles showed activity against all the tested pathogens. The time kill assay reveals that, the growth of the *Salmonella sp*. was inhibited by $ZrO_2$ from the 1st h onwards. It is concluded that, the $ZrO_2$ nanoparticles could be used as an effective antibacterial agent for the management of poultry systems. The $ZrO_2$ nanoparticle showed antibacterial activity against *Salmonella sp.* at a concentration of 5µg/ml. The time kill assay revels that, the bacterial growth was inhibited from the 1st h up to 12th h[47].

Kurantowicz et al., compared the toxicity of different forms of graphene family materials (GFM); pristine graphene (pG), graphene oxide (GO) and reduced graphene oxide (rGO) towards bacteria strains. The effect of three different GFMs on chosen food-borne bacteria strains: Gram-positive (G+) – *Listeria monocytogenes*, and Gram-negative (G-) – *Salmonella enterica* [48] was tested. Results are a decreased number of bacterial colonies observed in probes 250 µg/mL for all examined GFMs. Moreover, as low concentration of GO as 25 µg/mL caused a drop in the level of bacterial colonies as well and reduced growth by almost 100% [48].

T. Jin et al. [49] reported that the inhibitory efficacies of ZnO QDs against 3 pathogens (*Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli O157*) were concentration dependent and also related to type of application [49]. It was found that the treatment with ZnO formulation caused a net reduction in bacterial cells of 78% and 62% in the case of treated cotton and cotton/polyester fabrics while the net reduction in fungi was

calculated to be 80.7% and 32%, respectively [50]. ZnO is 1 of 5 zinc compounds that are currently listed as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (21CFR182.8991). The inhibitory effect of ZnO nanoparticles against *Salmonella* was dependent on the concentration of ZnO. After initial killing of cells by ZnO treatment, the cell populations of *Salmonella* remained constant during the 8 d incubation with the numbers of cells after 8 d at 5.5 log CFU/mL for 0.28 mg/mL. In the 1.12 mg/mL solution, *Salmonella* cells decreased to 3.5 log CFU/mL, whereas the control grew to 9.7logCFU/mL[49].

Antimicrobial growth inhibition and mechanistic activities of synthesized ZnO NPs were investigate from Navale et al. [51]. Nanoparticle size 20-25nm and concentration of 0, 20, 40, 60, 80 and 100μg/ml were used against pathogenic bacteria *S.aureus* (Gram positive) and *Salmonella thyphimurium* (Gram negative) and also first time against two plant fungi *Aspergillus flavus* and *fumigatus*. The growth analysis data indicated that the ZnO NPs have significant bactericidal effect on both bacteria. The quantity of died fungal biomass was negligible when the fungal culture was grown in presence of 100μg/ml NPs. The bactericidal effect was obtained at concentration 80μl/ml and bacteriostatic at 60μl/ml. These microbial analyses data indicates that ZnO NPs (size 20-25nm) have shown potential activity against these tested bacteria [51].

Antibacterial effects of ZnS: Ag nanoparticles against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* were investigated from Parvin et al.[52]. By increasing the ZnS concentration in wells and discs, the growth inhibition has also been increased. The size of inhibition zone was different according to the type of bacteria and the concentrations of ZnS:Ag QDs. Based on the diameter of inhibition zone for different bacteria the maximum inhibition activity is demonstrated against *Staphylococcus aureus* in comparison with *P. aeruginosa* and *S. typhi*. Data showed the similar results for different concentrations of ZnS nanoparticles antibacterial activity.The results of MICs obtained from broth dilution for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*, are 3.05 , 3.05 and 6.1 mg/ml whereas the amountsof obtained Minimal Bactericidal Concentrations are 12.2 , 6.1 and 12.2 mg/ml respectively [52].

Y. He et al., reported that magnesium oxide nanoparticles (MgO nanoparticles), with average size of 20 nm have considerable potential as antimicrobial agents in food safety applications due to their structure, surface properties, and stability [53].The observed antimicrobial effect was further investigated by exposing $10^8$ CFU/ml *C. jejuni* and $10^9$ CFU/ml *E. coli* O157:H7 and *S. enteritidis* to 0,5- 8 mg/ml MgO nanoparticles over a set time trial. Live cells were measured by the colony forming units onMH (Muler Hinton???) agar. At a concentration of 2 mg/ml MgO nanoparticles, *C. jejuni* was reduced 6 orders of magnitude after 2 h and completely killed after 4 h. At 4 mg/ml, *C. jejuni* was completely killed within 1 h. On the contrary, 8 mg/ml MgO nanoparticles were required to kill all *E. coli* O157:H7 and *S. enteritidis* cells in 4 h and 4 mg/ml in 6 h. In addition, *E. coli O157:H7* could also be killed by 2 mg/ml in 8 h, whereas *S. enteritidis* was only reduced 5 logs after the same exposure. This demonstrates again that MgO nanoparticles are effective at killing *C. jejuni* at low concentrations in short periods of time. They are also advantageous at killing *E. coli O157:H7* and *S. enteritidis* within 4 h [53].

Lima et al., in their work [54] used gold nanoparticles supported onto clinoptilolite, mordenite and faujasite zeolites. Content of gold in materials varied between 2, 3 and 2,8 wt%. The size, dispersion and roughness of gold nanoparticles were highly dependent of the zeolite support. The faujasite support was the support where the 5 nm NPs were highly dispersed. Gold nanoparticles dispersed on zeolites eliminate *E.coli* and *S.typhi* at short times. The biocidal properties of gold nanoparticles are influenced by the type of support which indeed, drives key parameters as the size and roughness of NPs. The most active material were pointed out as Au-faujasite. These materials contained particles size 5nm at surface and eliminate 90-95%of *E.coli* and *S.typhi* colonies for only90min. [54].

## Mechanism of action of nanoparticles against *Salmonella sp*

To explore antimicrobial mechanism of the nanoparticles, scanning electron microscopy was used to examine the morphological and membrane structure changes of *C. jejuni*, *E. coliO157:H7*, and *S. enteritidis* induced by MgO nanoparticles. Bacterial cells in late-log growth were treated with sub-lethal doses of MgO nanoparticles (1 and 2 mg/ml) for 4 h and collected for SEM study. Both treated and untreated cells were incubated under the same conditions and analyzed by SEM in parallel in order to observe the differences between the control and cells exposed to nanoparticles. SEM images show all of the untreated cells have intact and smooth surfaces. As expected, *C. jejuni* cells are spiral-shaped, whereas *E. coliO157:H7* and *S. enteritidis* are rod-shaped. After incubation with a sub-lethal concentration of nanoparticles, *C. jejuni* cells underwent significant morphological changes from spiral to coccoid form, but *E. coli O157:H7* and *S. enteritidis* remained rod shaped. Noticeably, all of the treated cells displayed some deep craters on their membrane surface, indicating a degree of membrane structure damage. These cells appear to be shorter and more compact, suggesting there could be some leakage of the cellular contents caused by the treatment. No cell lysis was noticed after the treatment of sub-lethal concentrations of nanoparticles [53].

The membrane permeability of *C. jejuni* after exposure to 1 and 2 mg/ml MgO nanoparticles for 4 h was assessed by EMA-qPCR assay. The results show that cells exposed to MgO nanoparticles had a nearly 1-log reduction in DNA amplification, indicating EMA penetration via damaged membranes. Similar experiments were performed on *E. coli* O157:H7 and *S. enteritidis* cells after exposure to 2 and 4 mg/ml MgO nanoparticles. The effects of membrane leakage by MgO nanoparticles were less noticeable compared to *C. jejuni* (data not shown). Together, these results indicate that MgO nanoparticles increase cell membrane permeability and that *C. jejuni* is more susceptible to the membrane damage than *E. coliO157:H7* and *S. Enteritidis* [53].

Krishnamoorthy et al., also reported for different mechanism of action in their work linked with antibacterial efficiency of graphene nanosheets against pathogenic bacteria via lipid peroxidation [55]. Graphene nanosheets are synthesized by a hydrothermal approach. The minimum inhibitory concentration

(MIC) of graphene nanosheets against pathogenic bacteria was evaluated by a microdilution method. MICs such as 1 μg/mL (against *Escherichia coli* and *Salmonella typhimurium*), 8 μg/mL (against Enterococcus faecalis), and 4 μg/mL (against *Bacillus subtilis*) suggest that graphene nanosheets have predominant antibacterial activity compared to the standard antibiotic, kanamycin. Measurement of free radical modulation activity of graphene nanosheets suggested the involvement of reactive oxygen species in antibacterial properties.

The free radical modulation activity of graphene nanosheets was determined using a lipid peroxidation assay [56]. Briefly, lipid peroxidation was induced in liposome prepared by ultrasonic irradiation from egg lecithin by adding 5 μL of 400 mM FeCl3 and 5 μL of 200 mM L-ascorbic acid. To this, the graphene nanosheets were added. A control which contained no compound was prepared. The samples were incubated at 37 °C for 60 min. The reaction was inhibited by adding 1 mL of stopping solution which contained 0.25 N HCl, 1.5% trichloroacetic acid, and 0.375% thiobarbituric acid. These reaction mixtures were kept in a boiling water bath for 15 min, cooled, and centrifuged. The absorbance of the resulting solution was measured at 532 nm.

Graphene nanosheets enhanced the ultrasound-induced lipid peroxidation. In comparison with the control group, lipid peroxidation was increased by 117% and 109% after exposure to 10 and 5 μg/mL of graphene ($p < 0.05$), respectively [55].

Berton et al., with the use of the transmission electron microscopy (TEM) were able to evaluate the interaction between Ag-NPs and two *Salmonella enterica* strains (*enteritidis* and *senftenberg*) and to study morphological changes caused by the interaction with nanoparticles.[57]The Ag-NPs appeared to interact rapidly with the two *Salmonella* serovar, adhering mainly to the cell wall. The interaction with the Ag-NPs resulted to be time limited in the case of *S. senftenberg*, while it was more long lasting for *S. enteritidis.*

Cell responses to Ag-NPs morphologically differed in *S. enteritidis* and *S. senftenberg*. Ag-NPs were adsorbed to the cell membrane of *S. enteritidis* and penetrated inside, thus modifying the cell structures. On the contrary, Ag-NPs were able to damage the cell wall of *S. senftenberg*, but did not enter the cells. These results show that the two *Salmonella* strains display different sensitivity to silver, with *S. senftenberg* exhibiting a resistant phenotype [57].

The response of *S. enteritidis* to Ag-NPs after 1 hour included: disruption of the cell wall, lysis of the cell membrane, damage of the cytoplasm and cell deformation. The interaction between Ag-NPs and the cell wall was characterized by the formation of "pits" and by their aggregation on the surface of the outer membrane thus determining an enhanced permeability of the bacterial membrane which allowed entry into the cell and, possibly, caused its death. In addition, electron dense Ag-NPs were found in cytoplasm of *S. enteritidis* and the damage of the cells displayed the formation of small electron lucent areas in cytoplasm. These regions appeared throughout the whole cell and aggregated in areas of high electron density located in the electron lucent cytoplasm. Another change induced by the adsorption of Ag-NPs was the widening of the periplasmic space, in which Ag-NPs had accumulated.

In the case of *S. senftenberg*, the disruption of the cell wall was observed after 1 hour, although the Ag-NPs did not penetrate inside and the authors did not detect any damage to the cell shape. After 4 hours, the *S. senftenberg* cells were mainly intact and maintained the same morphological structures as the control [57].

The study has demonstrated that Ag-NPs can be effective as an antimicrobial in the case of *Salmonella*, but its success is strongly strain-dependent, since differences in terms of time of action of AgNPs and sensitivity were observed for the two investigated serovars. This is probably due to genetic factors specifically intrinsic of each strain, including the presence of specific determinants of resistance, as demonstrated in the case of *S. senftenberg* [57].

In *Salmonella,* the silver resistance determinant present in some strains is encoded by genes located both on the plasmid and the chromosome. The silver determinant, studied on *Salmonella* plasmid pMG101, contains nine genes coding for one efflux ATPase (SilP), two metal-binding proteins (SilF and SilE), and one cation/proton antiporter (SilCBA). These proteins supposedly work in synergy: SilP releases Ag+ in the periplasmic space, SilF carries Ag+ from the periplasm to the inner membrane cation pump protein SilA, as a part of the SilCBA complex, which brings Ag+ out from the bacterial cell [58].

The shape and size of GFMs and their interactions towards bacteria strains were inspected by transmission electron microscope also. Bacteria were aggregated and attached to GFMs. A strong affinity occurred between bacteria and edges of pG and rGO, while bacterial strains attached to GO nanoparticle surfaces. The present results indicate that GFM antibacterial activity causes mechanical damage of bacterial cell membranes by a direct contact of the bacteria with the extremely sharp edges of GFM with $sp^3$-hybridized bonds. Based on the present results, the authors propose a three-step antimicrobial mechanism of GFM. It includes initial cell deposition on GFM (step 1), membrane stress and disruption caused by direct contact with sharp edges and bonds (step 2), and finally stimulated oxidation stress (step 3). The key difference between the chosen graphene materials is the bacterial cell deposition place [48].

The possible mechanism of action is, the metal nanoparticles are carrying the positive charges and the microbes are having the negative charges which create the electromagnetic attraction between the nanoparticles and the microbes. When the attraction is finished, the microbes get oxidized and die instantly [59]. Generally, the nano materials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface which leads to cell lysis [60].

## CONCLUSION

As a result of the survey we can conclude that nanobiotechnology is applicable at any stage of the fight against *Salmonella*. Many types of nanoparticles and nanocomposites give promising results on antibacterial effect evidence. It should be taken into account in future studies to clarify the mechanisms of action depending on factors such as concentration during treatment and strains dependence. From an overview, we can also mention trend for application of green synthesis of

nanoparticles that reduce environmental risk. We note also that when comparing the examined nanoparticles those with very low levels of MIC and MBC, should be a priority in future research.

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