EXPERT REVIEWS

Coping with antibiotic resistance: combining nanoparticles with antibiotics and other antimicrobial agents

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The worldwide escalation of bacterial resistance to conventional medical antibiotics is a serious concern for modern medicine. High prevalence of multidrug-resistant bacteria among bacteria-based infections decreases effectiveness of current treatments and causes thousands of deaths. New improvements in present methods and novel strategies are urgently needed to cope with this problem. Owing to their antibacterial activities, metallic nanoparticles represent an effective solution for overcoming bacterial resistance. However, metallic nanoparticles are toxic, which causes restrictions in their use. Recent studies have shown that combining nanoparticles with antibiotics not only reduces the toxicity of both agents towards human cells by decreasing the requirement for high dosages but also enhances their bactericidal properties. Combining antibiotics with nanoparticles also restores their ability to destroy bacteria that have acquired resistance to them. Furthermore, nanoparticles tagged with antibiotics have been shown to increase the concentration of antibiotics at the site of bacterium–antibiotic interaction, and to facilitate binding of antibiotics to bacteria. Likewise, combining nanoparticles with antimicrobial peptides and essential oils generates genuine synergy against bacterial resistance. In this article, we aim to summarize recent studies on interactions between nanoparticles and antibiotics, as well as other antibacterial agents to formulate new prospects for future studies. Based on the promising data that demonstrated the synergistic effects of antimicrobial agents with nanoparticles, we believe that this combination is a potential candidate for more research into treatments for antibiotic-resistant bacteria.

Keywords: antibacterial activity • antibiotics • combinations • nanoparticles

Frightening rise of antibiotic-resistant infections

Antibiotic resistance has become a serious global problem and affects not only intrahospital pathogens but also nonhospital strains for which treatment with antibiotics is available. According to an international multicenter study of antimicrobial resistance of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA) occurred with low levels in hospitals in Northern Europe (<1%), increasing levels in middle-European countries, the USA, New Zealand and Australia (6–22%), and very high levels in Southern European countries as well as in parts of the USA, Asia and South Africa (28–63%) [1].

Mainous *et al.* evaluated the trends in hospitilazations association with antibiotic-resistant infections in US hospitals between 1997 and 2006. This study revealed that antibiotic-resistant infection-related hospitalizations increased approximately 2.5-fold in the USA during that period [2].

Similiarly, the examination of the trends in prevalence of antibiotic-resistant hospital-acquired *Staphylococcus* spp. isolated from patients with thermal trauma in a Russian hospital between 2002 and 2008 showed two-fold increase in the number of MRSA and a 2.5-fold increase in the number of methicillin-resistant coagulase-negative staphylococci. The study also discovered a remarkable increase in the trends of staphylococcal resistance to aminoglycosides, macrolides, lincosamides, tetracyclines and fluoroquinolones [3]. In intensive care units of Russian hospitals between 2002 and 2004, the prevalence of extended-spectrum

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β-lactamase producers was on average 52.3% among enteric bacteria, 81.4% among *Klebsiella pneumonia* and reached up to 49.7% among *Escherichia coli* [4].

The Antibiotic Resistance Surveillance and Control in the Mediterranean region (ARMed) project, in which the prevalence of MRSA isolated from nine southern and eastern Mediterranean countries in 2003–2005 was examined, demonstrated that the overall median MRSA proportion was 39%. The highest levels of MRSA were observed in Jordan, Egypt, and Malta, which were all above 50%. In Turkey, prevalence of MRSA in Turkey was steadly decreased from 43 to 35% during the 3-year period [5]. More recent research demonstrated that MRSA prevalence was 50.2% in southeastern Turkey in 2007 [6]. In addition, in a study conducted by the European Antimicrobial Resistance Surveillance System in 2008, it was reported that MRSA prevalence among bloodstream-isolated *S. aureus* was more than 25% in one third of European countries [7]. It is obvious that the increased antibiotic resistance, which is one of the most important human health problems worldwide, seriously threatens more people every passing day. Therefore, there is an immediate need to develop new approaches to handle this problem.

Antibiotic resistance can be overcomed only by understanding its mechanisms. Major routes of antibiotic resistance include the efflux of antibiotic from the bacterial cell through efflux pumps, enzymatic modification or degradation of the antibiotic molecule, and alteration of the antibiotic target, which prevents binding of the antibiotic and, therefore, leads loss of its activity [8]. These various mechanisms suggest that the use of different approaches is required, including application of synergistic activity between antibiotics and nonantibiotics, inhibition of resistance enzymes that degrade or modify the antibiotic to a nonactive form, blocking antibiotic efflux or enhancing antibiotic entry into the cell and altering the physiology of the antibiotic insensitivity of cells [8]. However, the high prevalence of resistant bacteria makes the solution to this problem extremely difficult and necessitates novel approaches.

Nanoparticles: potential candidates for coping with drug-resistant bacteria

One of the promising approaches for overcoming bacterial resistance is the use of metallic nanoparticles [9–14]. Owing to their small sizes and higher surface-to-volume ratio, metallic nanoparticles have an enlarged contact area with a microorganism. This feature enhances biological and chemical activity, and therefore, acquires nanoparticles high antibacterial activity. Another important property of metallic nanoparticles is their ability to target different bacterial structures. Nanoparticles can disturb functions of cell membranes such as permeability and respiration. In addition, after penetrating into bacterial cell, nanoparticles can also disturb the functions of sulfur-containing proteins and phosphorus-containing compounds such as DNA by effectively reacting with them [15–16]. Complex action mechanisms of metals decreases the probability of bacteria developing resistance to them [17]. Although several resistance mechanisms to metals have been described, the most common, which is enhanced efflux of metal ions from the cell, is a high-level, single-step and target-based mutation. This mutation enhances efflux of metal ions from the cell and makes metal resistance less probable owing to its multifaceted mode of action [17]. This hypothesis was proved by the study that examined the frequency of spontaneous mutations to silver by *S. aureus*. Silver-resistant mutants were not detected when silver sulfadiazine was used as the selecting agent, indicating a frequency of mutations of less than 10^{-9} [18]. However, spontaneous mutations to a single antibiotic target are usually recorded with frequencies of approximately 10^{-8} [19].

In recent years, use of nanoparticles in antimicrobial applications have gradually increased. This increased interest in nanoparticles is illustrated by the number of publications on this topic (Figure 1). We reviewed publications using the search criteria 'antibacterial nanoparticles' on PubMed until 2010. Up until 1990, only 14 publications on this topic were found, and between 1990 and 1999 only 22. However, the number of publications has been steadly increased last ten years and reached to 255 in 2010 (Figure 1).

Owing to their high antibacterial properties, nanoparticles of silver, oxides of zinc, titanium, copper, and iron are the most commonly used nanoparticles in antimicrobial studies. Furthermore, these nanoparticles have been used to deliver other antimicrobial drugs to the site of pathological process [20]. Delivery of antibiotics by nanoparticles to the site of infection is a promising therapy particularly for controlled release of drugs, which in turn decreases the dose required to achieve a clinical effect [21]. For this purpose, biodegradable nanoparticles have been used mostly. Recent studies have revealed that metallic nanoparticles, such as gold nanoparticles, may also serve as a drug-delivery system. Gold nanoparticles are of great interest as drug and gene carriers. Furthermore, since gold nanoparticles can undergo a strong plasmon resonance with light, they are used for photo-activated drug release [22].

Although metallic nanoparticles exhibit significant antibacterial properties, they also possess serious disadvantages. Nanoscaled structures have demonstrated toxic action on some mammalian cells [23–25]. Wang *et al.* studied acute toxicity of oral exposure to nanoscale zinc powder in mice at a dose of 5 g/kg bodyweight and found pathological lesions in the liver, renal and heart tissue, and slight inflammation in the stomach. There were not any significant pathological changes in the rest of the organs [24]. Furthemore, nanoparticles of Fe_2O_3 increased microvascular permeability and cell lysis in lung epitheliums and disturbed blood coagulation parameters after intratracheal instillation [25]. In contrast to bigger particles, nanoparticles can be picked up by mitochondria and cell nuclei. These nanoparticles, once taken up by these organelles, may have the potential to cause DNA mutations [16]. However, in recent studies, combinations of nanoparticles with antibiotics, antimicrobial peptides and essential oils have been developed as promising approaches for reducing possible toxic effects of nanoparticles to the mammalian cells. Combined use of nanoparticles with antibiotics makes it possible to reduce the toxicity of both agents towards human cells due to decreased requirement for high dosages as well as synergistically enhancing their antimicrobial activities.

Combinations of antibiotics & antimicrobial peptides with metallic nanoparticles

Methods for studying interactions between nanoparticles & antibiotics

Classical methods used to assess interactions between two antimicrobial agents are disk diffusion, checkerboard, and time–kill curves [26]. Analysis of published studies has shown that the most commonly used method in studies of interactions between nanoparticles and antibiotics is disk diffusion, in which disks with antibiotics are further impregnated with some amount of nanoparticles (Table 1). Assessment of results in this method is performed by measuring enlargement of inhibition zone area around the disks, however, in some works interaction is assessed either by the percentage increase [27,28] or fold increase [29,30], whereas in other studies increased zone size is calculated simply as the difference between two zones [31,32]. These mild calculation disparities between methods of assessing the data make it more difficult to compare results of different studies. Another disadvantage of the disk diffusion method is difficulty in distinguishing between indifferent and synergistic interactions because of absence of such criteria [26]. All these limitations mean that the disk diffusion method is only presumptive in assessing antibacterial combinations.

Dilution methods are uncommon in studies of antibiotic–nanoparticle combinations. However, these methods may provide more information than the disk diffusion method and are less laborious than checkerboard and time–kill curves.

The checkerboard method provides more information about the interactions between antibacterial agents as it allows for investigation of combinations of different concentrations of nanoparticles and antibiotics. Furthermore, interpretation of results is more clear as there is a strict definition of synergy, indifference and antagonism through the use of fractional inhibitory concentration (FIC) index values, while in the disk diffusion method there are no such criteria. The checkerboard method is widely used in the study of antibiotic combinations, essential oil combinations, and antibiotic–essential oil combinations, but it is present only in a limited number of studies with nanoparticles [36]. This method also has several limitations, such as the fact that the standard checkerboard method does not examine bactericidal activity, and that calculation of FIC indexes assumes a linear dose–response curve for both agents so that when a dose–response is not linear, assessment of results becomes less obvious. The significant limitation of both the disk diffusion and checkerboard methods is that their time-independent view of interactions as results means that they can only be assessed at a single time interval [26].

Assessment of time-dependent nanoparticle-antibiotic interactions can be done by time–kill curves, which is widely used in the study of antibiotic interactions. However, this method is more cumbersome and laborious, and there are only a small number of studies in which time–kill curves were used to assess the antibacterial effect of nanoparticles alone [39,40] and of nanoparticle–antibiotic combinations [34]. However, application of time–kill curves to the study of interactions between nanoparticles and antibiotics will provide further information on the potential of such combinations for selecting resistant mutants.

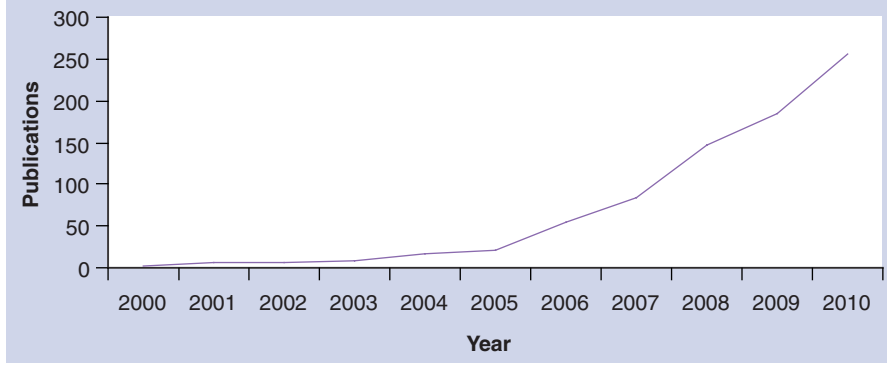

Figure 1. Dynamics of PubMed publications on antibacterial properties of nanoparticles. Data were obtained by performing a search of the PubMed database using the search criteria 'antibacterial nanoparticles' and included studies published up to 2010.

Combinations of silver nanoparticles with antibiotics & antimicrobial peptides

In recent years, several studies have been carried out on the subject of antibacterial efficacies of nanoparticle–antibiotic combinations. Investigation of the interactions of antibiotics with silver nanoparticles is the most common among studies dedicated to the examination of combined action of metallic nanoparticles with antibiotics. One of the earliest studies on this topic was published by Li *et al.* who investigated the combined activity of silver nanoparticles and amoxicillin against *E. coli* [34]. The authors determined the minimal inhibitory concentration (MIC) of amoxicillin and silver nanoparticles alone and in combination in Luria-Bertani medium. Results demonstrated low activity of silver nanoparticles and amoxicillin alone, but a significant reduction in bacterial growth by their combination. MICs for amoxicillin and silver nanoparticles were 0.5 mg/ml and 40 µg/ml, respectively. However, when they were combined, reduced quantities of these agents were required to achieve the same growth inhibition effect (0.150 mg/ml of amoxicillin and 5 µg/ml of silver nanoparticles). Authors also monitored dynamics of bacterial growth of *E. coli* over 20 h by measuring the optical density of the culture medium and transforming it into the number of CFUs in the presence of amoxicillin (0.15 mg/ml) and nanosilver (5 µg/ml) both separately and together. Combination significantly reduced bacterial growth in comparison with separate use of agents. Both a delayed and decreased duration of the exponential growth phase and a reduced stationary phase was observed.

The authors proposed different explanations for the synergistic effect between antibiotics and nanosilver for bacteria with and without antibiotic resistance. In resistant strains, enhanced activity can be explained by differences in the mechanism of action. If a bacterium exhibits resistance to one of the agents, another kills the microorganism in a different way. However,

Table 1. Methods used in the study of antibiotic–nanoparticle combinations.

Method	Short description	Advantages	Disadvantages	Ref.†
Disk or well diffusion	Standard disks with antibiotics are impregnated with nanoparticles and placed on inoculated medium. Instead of disks, wells in the medium can be made and a solution of antibiotic and nanoparticles is poured into the well. After incubation, the inhibition zone area around the disks with nanoparticles and antibiotics alone and in combination are compared	• Ease of performance • In the disk diffusion method, commercially available antimicrobial-impregnated disks with known antibiotic content can be utilized • Presentation of the observed effect as a fold or percentage increase of inhibition zone area allows statistical comparison between different combinations	• Results depend on diffusion of agents into the medium, therefore low diffusion may significantly change results • No criteria for distinguishing between a synergistic and indifferent effect • Bacteriostatic and bactericidial effects are not distinguished • Concentration-dependent interactions are not assessed • Time-dependent interactions cannot be studied	[27–33]
Dilution methods	Agar dilution: series of dilutions of antibiotics, nanoparticles and their combination in definite proportion are prepared in solid medium, which are then inoculated with bacteria. After incubation MICs of antibiotic, nanoparticle suspensions and their mixtures are compared Broth dilution (in recent studies, the microdilution method is more common): procedure is the same as agar dilution, but in liquid medium. By replacing content of tubes (or wells) without visual bacterial growth to the fresh medium without antimicrobial agent, the minimal bactericidal concentration is assessed	• Determination of MICs provides quantitative information by which comparison of activity of combinations and agents alone can be performed • Bacteriostatic and bactericidal effects can be assessed • Measuring optical density of tubes or wells in dynamics give preliminary information about time-dependent interactions between agents • In the agar dilution method several strains can be tested on one Petri dish which is useful in work with many strains simultaneously • Microdilution method deals with a small amount of antimicrobial agents which is useful in work with some antimicrobial agents present in small amount, for example, extracts or essential oils • Gives an opportunity to examine formation of resistance mutants by cultivation of bacteria in presence of sub-inhibitory concentrations of agents and determining MICs in dynamics	• Concentration-dependent interactions are not assessed • Time-dependent interactions are not usually studied	[34] [33,35]

†Indicates examples of references where the method was applied.
FIC: Fractional inhibitory concentration.

Table 1. Methods used in the study of antibiotic–nanoparticle combinations (cont.).

Method	Short description	Advantages	Disadvantages	Ref.[†]
Checkerboard	Series of dilutions of antibiotic and nanoparticles are prepared, mixed by pairs and inoculated with tested bacterium. After incubation, the MIC of each concentration combination is determined; this is used for calculation of the FIC index	• Concentration-dependent interactions between agents are assessed • There are strict criteria for defining synergy, indifference and antagonism	• Method is rather laborious • Calculation of FIC indexes assumes a linear dose-response curve • Time-dependent interactions are not assessed	[36]
Time–kill curves	Tested strain is incubated in liquid media with a definite concentration of antibiotic, nanoparticles and their combination. After different time intervals, some volume of medium is withdrawn, placed to solid medium and after incubation the number of CFUs is calculated. This procedure is performed several times to assess changes in CFU in dynamics	• Provides exact assessment of time-dependent interactions between agents	• The most cumbersome and laborious technique and because of this only few combinations can ever really be tested in the experiment	[34,37,38]

[†]Indicates examples of references where the method was applied.
FIC: Fractional inhibitory concentration.

this mechanism is more obvious for indifferent or additive action rather than for synergy. In the bacterial strains without resistance, enhanced effect was explained by the binding reaction of amoxicillin and nanosilver. Hydroxy and amido groups in the molecule of amoxicillin react easily with nanosilver by means of chelation (Figure 2). Antimicrobial groups together with nanosilver produce a structure made up of a nanosilver core surrounded by amoxicillin molecules. It is assumed that the antimicrobial groups act on one point at the bacterial cell surface and cause more destruction by increasing the concentration of antimicrobial agents. Since silver chelation prevents DNA from unwinding, nanosilver's antimicrobial groups cause more dramatic DNA damage. Durán *et al.* further discussed the binding reaction between amoxicillin and silver nanoparticles and attributed the enhanced activity to sulfur bridges in the molecule of amoxicillin, which are the most important binding sites [41]. Another important explanation given by Li *et al.* is the role of nanosilver as a carrier for an antibiotic [34]. Unlike hydrophilic amoxicillin, nanosilver is hydrophobic and, therefore, is more ready to interact with a bacterial membrane composed of hydrophobic phospholipids and glycoproteins. This feature of nanosilver facilitates transportation of amoxicillin to the cell surface.

Interactions between different antibiotics and silver nanoparticles produced by bacteria and fungi were investigated in several studies [27,29,30]. In these studies nanoparticles were produced biosynthetically, either by *Klebsiella pneumoniae* [29], or by fungi *Trichoderma viride* [27] or *Phoma glomerata* [30].

Shahverdi *et al.* impregnated standard antibiotic disks with 10 µl of silver nanoparticles of average size 22.5 nm (range: 5–32 nm) at a final content of 10 µg per disk by using the disk diffusion method [29]. Results were assessed by measuring fold increases in the area of inhibition zone. Combined action of silver nanoparticles with 14 antibiotics from different groups (penicillin G, amoxicillin, carbenicillin, cephalexin, cefixime, gentamicin, amikacin, erythromycin, tetracycline, cotrimoxazole, clindamycin, nitrofurantoin, nalidixic acid and vacomycin) was examined against Gram-positive and Gram-negative bacteria – *S. aureus* and *E. coli*, respectively. The results of the study showed enhancement of antibacterial action of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin in the presence of silver nanoparticles against both tested strains. However, in general, effects of silver nanoparticles on the activity of antibiotics against *E. coli* were lower than against *S. areus* (Figure 3A). It was observed that fold increases in area ranged from 0.7 to 2.5 against *S. aureus* and from 0.4 to 1.3 against *E. coli*. Effect was maximum with vancomycin, amoxicillin, and penicillin G against *S. aureus* (fold increase in area was 2.5 for both vancomycin and amoxicillin and 1.9 for penicillin G), while the highest enhanced activity was observed in erythromycin and penicillin G against *E. coli* (fold increase in area was 1.3 for both antibiotics). Mechanisms of either synergistic or indifferent interactions were not proposed in the study. However, the highest enhanced effect of penicillins and differences in the interaction levels between Gram-positive and Gram-negative bacteria indicated the key role of the bacterial cell wall, which is further discussed in the following studies.

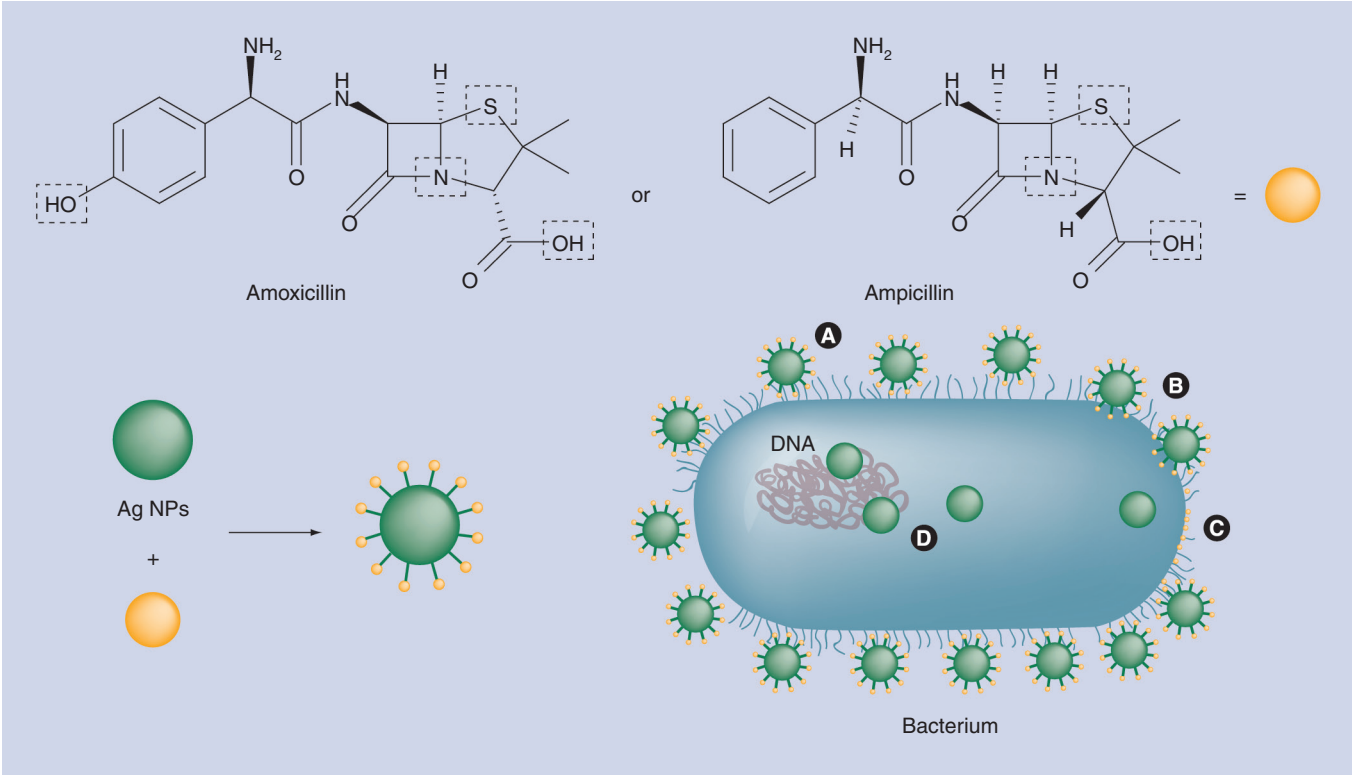

Figure 2. Mechanism of interaction between amoxicillin or ampicillin and silver nanoparticles. Silver nanoparticles produce a complex with amoxicillin or ampicillin (possible binding sites are indicated within dotted lines); bonding reaction between antibiotic and silver nanoparticles increases the concentration of antimicrobial agents at the bacterial surface **(A)**; the silver nanoparticles act as antibiotic carrier and facilitate approach of the hydrophobic antibiotic to the bacterial cell **(B)**; penicillin antibiotics destroy the cell wall and increase its permeability for silver nanoparticles **(C)**; silver nanoparticles prevent the DNA from unwinding **(D)**.
Ag NP: Silver nanoparticle.
Adapted from [27,34].

Fayaz *et al.* investigated combined antibacterial effects of silver nanoparticles with four different antibiotics (ampicillin, erythromycin, kanamycin and chloramphenicol) against two Gram-positive (*S. aureus* and *Micrococcus luteus*) and two Gram-negative (*E. coli* and *Salmonella typhi*) microorganisms [27]. Antibacterial activity of all tested antibiotics increased in the presence of silver nanoparticles of 5–40 nm [29] (more abundant were nanoparticles of 5–15 nm) at a content of 10 µg per disk. However, in contrast to the study by Shahverdi *et al.*, the increase was more prominent against Gram-negative bacteria than against Gram-positives for all antibiotics. The highest enhancing effect was observed between silver nanoparticles and ampicillin, and the percentage of fold increase of inhibition zone areas in ampicillin with silver nanoparticles against both groups of bacteria was almost equal (Figure 3B). The MIC of silver nanoparticles alone was also low against Gram-positive bacteria. Results were explained by the differences in cell wall structures of Gram-positive and Gram-negative bacteria. As is known, the cell wall in Gram-positive bacteria is composed of a thick layer (~20–80 nm) of peptidoglycan, which consists of linear polysaccharide chains cross-linked by short peptides, thus forming a 3D rigid structure. In Gram-negative bacteria the layer of peptidoglycan is thinner (~7–8 nm) with an external layer of lipopolysaccharides which are not rigid. The rigidity and cross-linking of the Gram-positive cell wall is associated with endowing the cell wall with fewer anchoring sites for silver nanoparticles and thus, results in difficulties in penetration. The high enhancing effect between ampicillin and silver nanoparticles is explained by lysis of cell wall caused by ampicillin and an increase in cell wall penetration for silver nanoparticles [27].

Birla *et al.* studied the combined effect of silver nanoparticles with five antibiotics (ampicillin, gentamicin, kanamycin, streptomycin and vancomycin) on the three most common human pathogens – *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* [30]. In contrast to the study of Shahverdi *et al.* [29] and in agreement with Fayaz *et al.* [27], activity of antibiotics in the presence of silver nanoparticles at a content of 15 µl per disk increased more significantly against Gram-negative bacteria for all antibiotics, except streptomycin. It was observed that after exposure to a combination of silver nanoparticles and ampicillin, fold increase in area was 1.8 and 2.4 against *E. coli* and *P. aeruginosa*, respectively, while it was only 0.2 against *S. aureus*. For streptomycin, fold increase in area was 0.6 against *S. aureus* but 0.3 against *E. coli*, while against *P. aeruginosa* it was 1.8. The highest enhancing effect was observed for vancomycin: the fold increase in area was 2.4 against both *E. coli* and *P. aeruginosa* and 0.2 against *S. aureus* (Figure 3C). Because a high enhancing effect was observed in vancomycin and ampicillin, which both target

the cell wall of bacteria, it may be explained by an increase in penetration of silver nanoparticles through the cell membrane in the presence of these antibiotics.

Ruden *et al.* studied interactions between silver in forms of silver nitrate and silver nanoparticles of mean diameter 25 nm and different antimicrobial peptides (polymyxin B, gramicidin A, alamethicin, gramicidin S, PGLA and magainin 2) [36]. The authors used nine strains of bacteria: *E. coli*, *Acinetobacter calcoaceticus*, *Enterobacter helveticus*, *Aeromonas bestiarum*, *Proteus myxofaciens*, *Pseudomonas fluorescens*, *Bacillus subtilis*, *Kocuria rhizophila* and *Micrococcus luteus*. Interactions between silver compounds and antimicrobial peptides were studied using a 2D microdilution assay (checkerboard method) with determination of MICs and calculation of FIC index, where FIC index values lower than 0.5 indicate synergistic effect. Polymyxin B demonstrated synergy action with silver nanoparticles against five tested strains – all Gram-negative bacteria (FICs were in ranges from 0.23 against *P. myxofaciens* to 0.39 against *A. bestiarum*), while with $AgNO_3$ synergy was noticeable only against *P. myxofaciens*, *E. helveticus*, and *E. coli* (FICs were 0.23, 0.29 and 0.38, respectively). Gramicidin S showed synergy with silver nanoparticles against *P. myxofaciens*, *E. helveticus* and *P. fluorescens* (FICs were 0.38, 0.44 and 0.48, respectively), and with $AgNO_3$ only against *E. helveticus* (FIC was 0.32). All other combinations showed only an additive inhibition effect. *A. calcoaceticus* did not demonstrate sensitivity to silver nanoparticles even at the highest concentration (1024 µg/ml). The authors concluded that, in general, silver nanoparticles had more synergistic effect with antimicrobial peptides in contrast to silver ions. The researchers not only studied synergistic interactions between antimicrobial peptides and nanoparticles but also proposed the mechanism of these interactions. They suggested that polymyxin B increases permeabilization of the outer bacterial membrane thus, enhancing the intrinsic antibiotic effect of the silver nanoparticles.

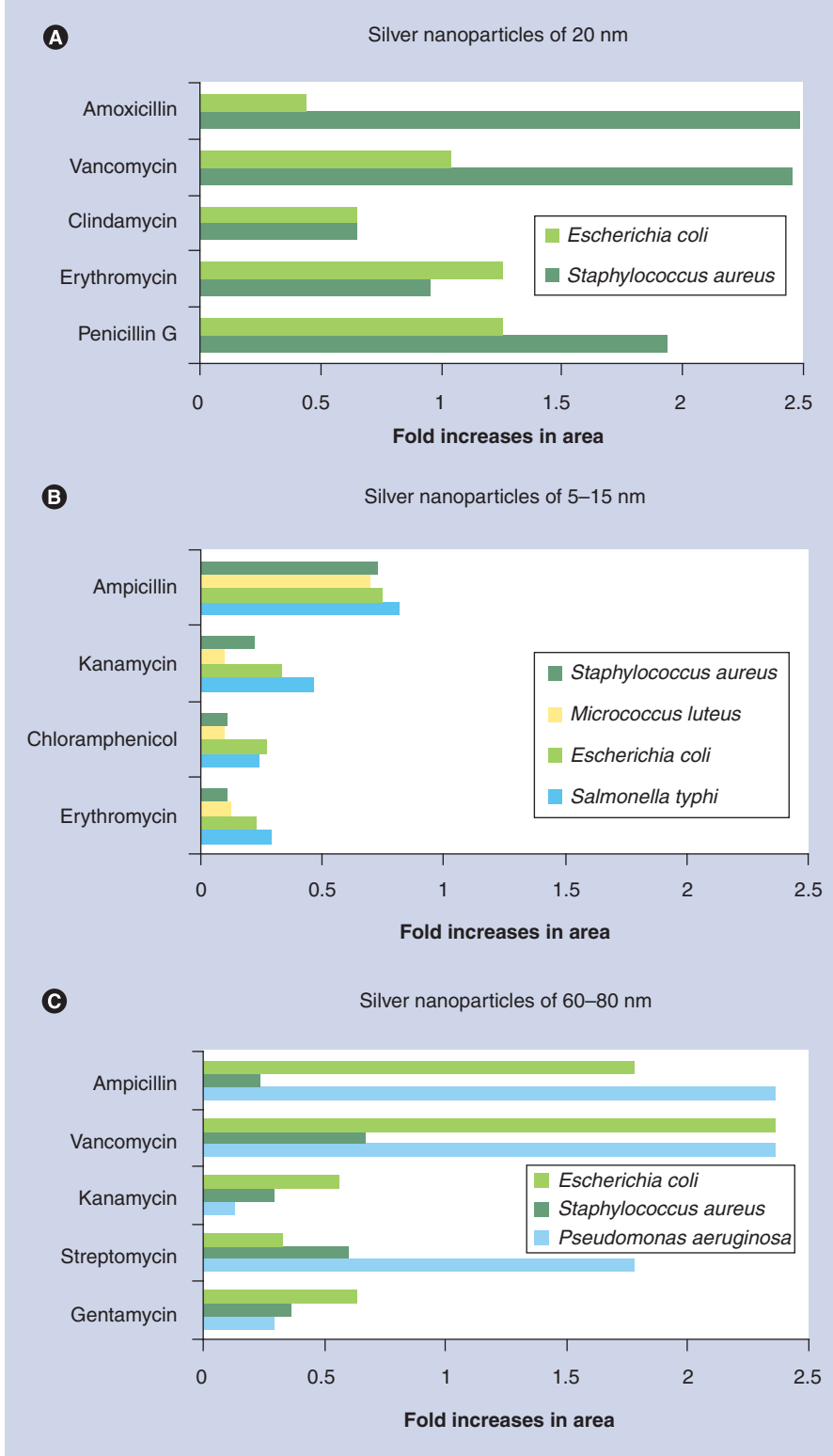

Figure 3. Fold increases in area of inhibition zones around the disks with silver nanoparticles of different sizes and different antibiotics compared with inhibition zones of antibiotics alone.
(A) Data taken from [29]. **(B)** Data taken from [27]. **(C)** Data taken from [30].

Combinations of titanium dioxide nanoparticles with antibiotics

There have been studies in which interactions between other types of metallic nanoparticles and antibiotics have been assessed. One of the commonly used nonsilver nanoparticles is titanium dioxide (TiO_2). Antibacterial action of TiO_2 nanoparticles is photodependent [42–49]. Mechanism of

bactericidal effect is attributed to the generation of free radicals during photocatalytic reactions. Free radicals affect lipopolysaccharide (LPS), peptidoglycan and phospholipids bilayer owing to peroxidation. Li *et al.* compared the antibacterial activities of TiO_2 nanoparticles, ultraviolet (UV) radiation, and combined action of these factors [34]. Results of the work demonstrated that combined action of the two factors had the most significant damaging effect to the outer membrane of *E. coli*. TiO_2 alone or UV radiation could break down the outermost LPS layer of *E. coli* cells but did not destroy peptidoglycan. After exposure to TiO_2 or UV light bacterial cells became twisted and rougher. Authors noticed that damages were in regular wrinkles and had the appearance of groove-like rifts. Cells bulged at both ends; their shape became wider and shorter but in general retained the rod-shaped form. Theses changes in bacterial shape and roughening of cell surface indicated ruptures of outermost layer of the cell to some extent but peptidoglycan was not damaged. After exposure to TiO_2 along with UV light, cells completely lost their rod shape and became elliptical with production of spheroplasts. These findings indicated destruction of all layers of outer membrane including LPS, peptidoglycan and a phospholipid layer. Since the inner membrane still existed, bacterial cells were still alive and did not break apart [49]. Without photo-activation, TiO_2 nanoparticles themselves did not show any noticeable antibacterial activity. Under normal laboratory lighting in liquid growth conditions TiO_2 nanoparticles lead to an approximately 20% growth reduction of *S. aureus* [50].

Understanding the mechanism of action of TiO_2 nanoparticles is important in the study of their interactions with antibiotics. Activities of different antibiotics with TiO_2 nanoparticles of approximately 20 nm against MRSA were studied by Roy *et al.* [31]. The authors studied activities of antibiotics such as penicillins, cephalosporins, glycopeptides, aminoglycosides, fluoroquinolones, azalides, macrolides, lincosamides and sulfonamides. Results of interactions between nanoparticles and antibiotics were assessed by the linear increase in zone inhibition sizes. In the presence of subinhibitory concentrations of TiO_2 nanoparticles (10 µg/disk), the inhibition zone areas were increased maximally around the disks with penicillin and amikacin (10 mm) followed by ampicillin and gentamycin (in each 9 mm), oxacillin and cloxacillin (8 mm), amoxycillin, cephalexin, cefotaxime, ceftazidime, vancomycin, streptomycin (in each 7 mm), erythromycin, clindamycin (6 mm), and tetracycline (5 mm). The moderate increases in the areas of inhibition zone were characteristic for ciprofloxacin, rifampicin, sulphazidime and cotrimoxazole (4 mm). The lowest increase in inhibition zone areas was detected for chloramphenicol (3 mm) followed by norfloxacin and clarithromycin (2 mm). With nalidixic acid TiO_2 nanoparticles showed no beneficial antibacterial effect. The authors made the conclusion that at the tested concentrations, TiO_2 nanoparticles significantly improved antibiotic efficacy against *S. aureus* in combinations with penicillins, cephalosporins and aminoglycosides. Although the authors demonstrated promising activity of different antibiotics in combinations with TiO_2 nanoparticles against MRSA, the mechanism of these interactions was not discussed. However, results of the study, especially the high enhancing effect between penicillins and TiO_2 nanoparticles, are similar to other studies that were conducted with silver nanoparticles. This indicates the possibility that they may use the same mechanism to enhance interactions – adverse action on the cell wall by penicillin antibiotic which increases penetration of metallic nanoparticles. Enhancing activity of antibiotics with another mechanism of action, for example, aminoglycosides or macrolides, which inhibit protein synthesis in the bacterial cell, merits further investigation. This study used normal laboratory conditions without any irradiation [31]. However, taking into account the photodependent activity of TiO_2 nanoparticles, it may also be useful to study antibiotic–TiO_2 nanoparticle combinations with UV radiation.

Combinations of zinc oxide nanoparticles with antibiotics

Influence of zinc oxide (ZnO) nanoparticles on the antibacterial activity of 25 different antibiotics against *S. aureus* was studied in the research of Thati *et al.* using the disk diffusion method [32]. In the presence of a subinhibitory concentration of ZnO nanoparticles of 80 ± 5 nm (100 µg/disk) the highest increases in the inhibition zones were observed for penicillin, amoxicillin/clavulanic acid and amikacin (10 mm), followed by methicillin, oxacillin, ampicillin, amoxicillin, gentamicin (9 mm), cloxacillin (8 mm), cephaxine, cefotaxime, ceftazidime, vancomycin, streptomycin (7 mm), erythromycin, clindamycin (6 mm) and tetracycline (5 mm). The moderate increases in inhibition zones were present in ciprofloxacin, rifampicin, sulphazidime, and cotrimoxazole (4 mm). The lowest increase in inhibition zones were characteristic for chloramphenicol (3 mm) followed by norfloxacin and clarithromycin (2 mm). Similar to the study with TiO_2 nanoparticles, nalidixic acid with ZnO nanoparticles showed no enhancing effect in the antibacterial activity. Therefore, the results of the research demonstrated that ZnO nanoparticles can potentiate antibacterial efficacies of penicillins, cephalosporins, aminoglycosides, glycopeptides, macrolides, lincosamides and tetracycline. Although results demonstrated enhancing influence of ZnO nanoparticles on the activity of many antibiotics against *S. aureus*, mechanisms of these interactions were not discussed and therefore require further investigation.

Combined activity of ZnO nanoparticles (20–45 nm) with different antibiotics against *S. aureus* and *E. coli* was also studied by Banoee *et al.* using the disk diffusion method [28]. Assessment of results was done by calculating the percentage of increase in inhibition zone areas for antibiotics in the presence of nanoparticles. In contrast to the results of Thati *et al.* [32] and to the studies with silver nanoparticles, ZnO nanoparticles in the present study demonstrated antagonistic influence on activity of amoxicillin. According to the results, the inhibition zone area decreased by 36% in the presence of ZnO nanoparticles (500 µg/disk) against *S. aureus*. The results also showed a decrease of activity of penicillin G, nitrofurantoin, vancomycin and carbenicillin against *S. aureus* in the presence of ZnO nanoparticles. Against *E. coli*, ZnO nanoparticles also acted antagonistically in the combination

with erythromycin: the inhibition zone area decreased by 12.9%. The only antibiotic that demonstrated beneficial interactions with ZnO nanoparticles was ciprofloxacin: in the presence of ZnO nanoparticles a total of 27 and 22% increase in inhibition zone areas was observed against *S. aureus* and *E. coli*, respectively. The authors then studied the effect of ZnO nanoparticles on the activity of ciprofloxacin in three concentrations (500, 1000 and 2000 µg/disk) against different clinical isolates. The results of this study showed a concentration-dependent effect of ZnO nanoparticles on antibacterial properties of ciprofloxacin with the highest enhancing activity of nanoparticles in the content of 2000 µg/disk. The authors proposed an explanation for the increased activity of ciprofloxacin in the presence of ZnO nanoparticles against *S. aureus*. ZnO nanoparticles may interfere with the pumping activity of NorA protein by inducing faster electron transfer kinetics in its active site. This protein mediates the active efflux of hydrophilic fluoroquinolones from the bacterial cell providing the resistance to the bacterium and this lead to inhibition of antibacterial activity of ciprofloxacin. Interference with NorA protein restores ciprofloxacin action. Another suggested mechanism is explained by the interactions of ZnO nanoparticles with membrane Omf protein, which is associated with permeation of the cell membrane by quinolones. In this way, ZnO nanoparticles enhance ciprofloxacin absorption into the cell. The ciprofloxacin molecule has three amino groups together with the electron-donor fluore group (Figure 4). The authors suggested that just this unique chemical feature had an important role in fairly strong interactions with ZnO nanoparticles. The fluore group may interact with the chelating Zn atom stabilizing the ciprofloxacin–nanoparticle combination. Another important group in ciprofloxacin, which interacts with the Zn atom, is the carboxyl group, which serves as an obvious target for chelation by metal ions. On the other hand, decreased or unchanged antibacterial activity of the other tested antibiotics was explained either by formation of weak hydrogen bonds with hydroxylated ZnO nanoparticles or by lack of sufficient targets for interaction.

Mechanisms of ZnO nanoparticle–ciprofloxacin interactions proposed by Banoee *et al.* can be extrapolated to the other metallic nanoparticles and antibiotics also. Efflux pump systems are responsible for the resistance to many antibacterial agents, such as fluoroquinolones, macrolides, aminoglycosides, tetracyclines and more. In both Gram-positive and Gram-negative bacteria [51]. Ability to modify electron transfer kinetics in active site of enzymes was also described for TiO_2 [52], therefore, similar to ZnO nanoparticles, TiO_2 nanoparticles may influence these efflux pumps and prevent antibiotic resistance.

Combinations of gold nanoparticles with antibiotics

Gold nanoparticles are generally regarded as being biologically inert [53] and antibacterial activity is not characteristic of gold nanoparticles themselves [54]. However, several studies demonstrated an enhanced effect of some antibacterial agents in the presence of gold nanoparticles, although the detailed mechanism of such an effect is not still well understood.

Chamundeeswari *et al.* showed twofold enhancement of antibacterial activity of chitosan-capped gold nanoparticles coupled with ampicillin compared with activity of ampicillin alone [55]. Perni *et al.* demonstrated the ability of gold nanoparticles to increase photodependent oxidative action of methylene blue embedded in polysiloxane polymers on MRSA and *E. coli* [37]. Concentrations of singlet oxygen produced during irradiation at, or close to, the polymer surface were similar in polymers containing methylene blue with and without gold nanoparticles. Owing to this, the authors concluded that action of gold nanoparticles is due to enhancement of the light-induced antimicrobial activity of methylen blue by a possible increase in the yield of reactive oxygen species other than singlet oxygen.

According to findings of Zhao *et al.* amino-substituted pyrimidines, which themselves do not possess any antibiotic action, in the presence of gold nanoparticles exhibit antibacterial activity against multidrug-resistant clinical isolates (e.g., *E. coli*, *P. aeruginosa*) without any additional source of energy such as light irradiation [35]. In addition to these findings, the authors also gave an explanation of antibacterial action, according to which pyrimidine-capped gold nanoparticles caused sequestration of magnesium or calcium ions leading to disruption of the bacterial cell membrane. This, in turn, results in leakage of cellular

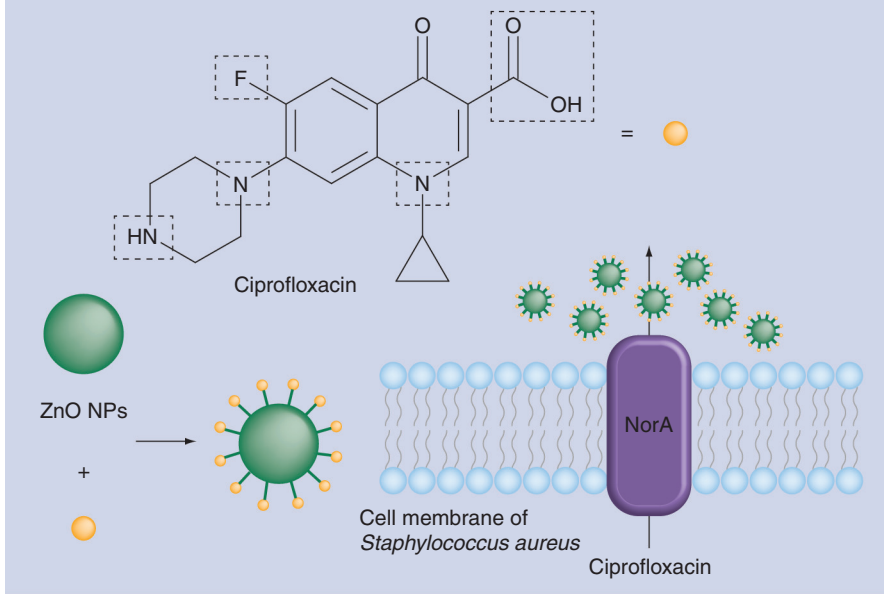

Figure 4. Mechanism of interaction between ciprofloxacin and zinc oxide nanoparticles. ZnO nanoparticles produce complex with ciprofloxacin (possible binding sites are indicated within dotted lines) and interfere with pumping activity of NorA protein of *Staphylococcus aureus* cell membrane thus inhibiting ciprofloxacin efflux from the cell. NP: Nanoparticle; ZnO: Zinc oxide.
Adapted from [28].

content including nucleic acids. Furthermore, internalized nanoparticles lead to DNA and protein synthesis inhibition, and amino-substituted pyrimidine-capped gold nanoparticles have been shown to induce resistance more slowly compared with conventional, small-molecule antibiotics. The authors compared changes in MICs after exposure of *E. coli* to different concentrations of 4,6-diamino-2-pyrimidinethiol (DAPT)-capped gold nanoparticles with changes in MICs of gentamicin. The MIC of DAPT-capped gold nanoparticles slightly increased (from 6 to 8 µg/ml) after 21 passages in the presence of two-thirds of the MIC, and did not change even after next 30 passages. By contrast, *E. coli* quickly developed resistance to gentamicin and became insensitive to the original MIC even after one passage, while after ten passages MIC increased by ten times (from 1 to 10 µg/ml) in the presence of increasing concentrations from two-thirds of the MIC [35].

However, the results of the study by Burygin *et al.*, who studied antibacterial action of gentamicin in the presence of 15-nm colloidal-gold particles on *E. coli*, demonstrated absence of significant differences between antibacterial activity of pure gentamicin and its mixture with gold nanoparticles [33]. The authors studied the activity of a mixture of gentamicin and colloidal-gold particles toward *E. coli* by using the agar-well diffusion method, enumeration of CFUs and turbidimetry. In the disk diffusion method, gold nanoparticles themselves did not possess antimicrobial activity and increasing concentrations of gold nanoparticles (0.1, 0.5 and 1.0 mM) mixed with gentamicin did not influence the activity of the antibiotic. Because addition of antibiotic caused aggregation of gold nanoparticles, the authors suggested that absent activity could be due to the inability of these aggregated particles to penetrate into agar gel. Absence of penetration into the agar gel was proved experimentally; however, in liquid medium, gold nanoparticles also did not influence activity of antibiotic, and the same results were also obtained for bactericidal activity detected by counting CFUs. The authors emphasized that their findings were in conflict with earlier findings that indicated an enhancement of antibacterial activity of similar mixtures of gentamicin with gold nanoparticles, and suggested that at least two conditions were necessary for enhancing activity of antibiotics using gold nanoparticles. The first was preparation of stable conjugates of nanoparticles coated with the antibiotic molecules. The second condition was a large amount of antibiotic covering the bacterial surface to efficiently increase the local antibiotic concentration at the site of contact with the bacterium. In this way gold nanoparticles may act as drug carriers owing to an increase in antibiotic concentration in the bacterial proximity.

Promising combinations: nanoparticles & essential oils

Another promising approach in coping with antibiotic resistance is the use of natural antimicrobial substances such as essential oils of plants [56–58]. Essential oils, besides their high antimicrobial activity, have demonstrated antioxidant, anti-inflammatory, immune modulatory, regenerative and other beneficial properties [59–61]. Although essential oils and their antimicrobial properties have been known for a long time, widespread antibiotic resistance restored an interest to them, which nowadays is widely demonstrated by a significant amount of publications on antibacterial properties of different essential oils and their components [62]. There are also some works which study interactions of essential oil components with polymeric nanoparticles used for delivering these oil components into the site of infection [63,64].

Chen *et al.* prepared nanoparticles with antibacterial and antioxidant activity by grafting two components of essential oils, eugenol (phenylpropene, which is the component of clove, cinnamon, basil and other essential oils) and carvacrol (monoterpenoid phenol, the component of oregano, thyme, and other oils), on chitosan nanoparticles [63]. The authors assessed antibacterial activity of nanoparticles against *E. coli* and *S. aureus*, and demonstrated that the grafted eugenol and carvacrol conferred antioxidant activity to the chitosan nanoparticles, and antibacterial activity of these grafted nanoparticles was better or equal to activity of unmodified chitosan nanoparticles. The authors also studied cytotoxicity of these nanoparticles using 3T3 mouse fibroblasts, and found that toxicity of eugenol-grafted chitosan nanoparticles and carvacrol-grafted chitosan nanoparticles was significantly lower than cytotoxicity of pure essential oils [63].

Hu *et al.* synthesized chitosan nanoparticles grafted with thymol, the natural monoterpene phenol, which is the main component of thyme, oregano, ajowan and other essential oils [64]. Results of the research illustrated that thymol-loaded water-soluble chitosan nanoparticles had stronger antibacterial activity than thymol itself. Furthermore, a decrease in the size of these nanoparticles demonstrated a stronger antimicrobial effect on Gram-positive bacteria and fungi. The MICs of nanoparticles were 0.00313–0.00157% (w/v) against *S. aureus* and *Bacillus subtilis* [64].

Therefore, both studies have demonstrated rationality of preparing chitosan nanoparticles grafted with essential oil components for increasing antibacterial and antioxidant activity, and for reduction of toxic effect. However, in contrast to traditional antibiotics, literature overview demonstrates an absence of research devoted to the study of combinations of essential oils or their components with metallic nanoparticles. Nanoparticle–essential oil combinations are not applicable to the development of antibacterial coatings or materials because of volatile characteristics of essential oils; but they may be promising agents in the treatment of topical infections of any location owing to the presence of not only antimicrobial properties in both agents but also different healing capacities of essential oils.

Activity of nanoparticles in combinations with antibiotics against multidrug-resistant bacteria

Different nanoparticles have been shown to be effective against multidrug-resistant bacteria: silver nanoparticles demonstrated activity against MRSA and MRSE [65], vancomycin-capped gold nanoparticles were active against vancomycin-resistant enterococci (VRE) [38]; and polysiloxane polymers containing embedded methylene blue and gold nanoparticles under exposure to 660-nm laser radiation had good activity against MRSA [35]. One

of the suggested mechanisms of increased activity of nanoparticles against multidrug-resistant bacteria is achieving a relatively higher concentration of the antimicrobial agent at the site of bacteria–nanoparticle contact [21].

Gu *et al.* proposed a way of overcoming bacterial resistance using gold nanoparticles coated with vancomycin [38]. Vancomycin binds to D-alanine repeat units (D-ala-D-ala) on the bacterial surface and then inhibits biosynthesis of peptidoglycan of the cell wall. However, in vancomycin-resistant bacteria (e.g., VRE) terminal cell-surface peptides (such as D-lactate) undergo modifications and this lowers vancomycin activity [66]. Gold nanoparticles possess not only small sizes (3–5 nm, which is a thousand times smaller than a bacterium), but also maintain a constant shape and size in solution, which makes them a good model for study of multivalency [38]. In this study, gold nanoparticles coated with vancomycin were prepared through the interactions of synthetic gold nanoparticles with bis(vancomycin) cystamide, and these nanoparticles, through gold and sulfur interactions, demonstrated increased activity of vancomycin against VRE with resulting MICs of 2–4 µg/ml [38]. A possible mechanism for this can be explained by the formation of close contacts among molecules of vancomycin (~31 per nanoparticle) leading to changes in binding properties by multivalent inhibition [66,67]. Vancomycin-capped gold nanoparticles may interact in a multivalent way with amino acid residues of the glycanpeptidyl precursors on glycosides of bacterial membrane (Figure 5) [38].

Vancomycin has been used for the treatment of MRSA infections since 1980, however, in recent years vancomycin-resistant *S. aureus* (VRSA) has appeared, the first of which was registered in 2002 in the USA. Several therapies for low-level vancomycin-resistant (vancomycin-intermediate *S. aureus*) and VRSA are recommended, one of which is linezolid; however, mutations of 23S ribosomal RNA genes may also lead to linezolid resistance [68]. One of the proposed approaches to overcome bacterial resiatnce is the use of folic acid-tagged chitosan nanoparticles which directly deliver vancomycin into the bacterial cell [69]. Another method was proposed by Fayaz *et al.*, who synthesized gold nanoparticles coated with vancomycin by bonding vancomycin to the surface of biosynthetically produced gold nanoparticles through ionic interactions between positively charged amine groups of vancomycin and negatively charged surface of gold nanoparticles [70]. These nanoparticles demonstrated activity against VRSA at a MIC of 8 µg/ml, which was explained by possible nonspecific binding of nanoparticles to cell surface peptides that were involved in the synthesis of the cell wall.

Furthemore, gold nanoparticles coated with vancomycin were shown to be active against *E. coli*, which is normally resistant to vancomycin owing to its inability to penetrate the outer membrane of Gram-negative bacteria [38,70]. Owing to presence of pits in the cell membrane under transmission electron microscopy, a possible mechanism of activity against *E. coli* is penetration of the Gram-negative bacterial membrane [70]. Therefore, gold nanoparticles facilitate binding of vancomycin to the bacterial cell surface independent of its structure in both Gram-positive and Gram-negative bacteria.

Results of different studies on combinations between nanoparticles and antibiotics are summarized in the Table 2.

Expert commentary & five-year view

In spite of the presence of different methods proposed to combat microbial resistance, the high prevalence of multidrug-resistant bacteria indicates an urgent requirement for new approaches to cope with this problem. High biological and chemical activity of metallic nanoparticles makes them promising agents in antibacterial treatment. However, current usage of metallic nanoparticles is restricted because of their toxicity.

Owing to combined use of antibiotics with metallic nanoparticles some previously effective antibiotics can be restored, for example some penicillins [27,29,30,34], and also emerging resistance to presently highly effective antibiotics, such as vancomycin, can be overcome [38]. In addition, combined use of nanoparticles with antibiotics or other antimicrobial agents makes it possible to reduce toxicity of both agents towards human cells [63].

The majority of studies are devoted to the study of interactions between antibiotics and silver nanoparticles, and many combinations have shown a promising enhancing effect *in vitro*, mainly with β-lactams (ampicillin, amoxicillin) and glycopeptides (vancomycin). These results are most probably due to an increase in cell wall penetration by these antibiotics for the nanoparticles. Although other metallic nanoparticles are not yet widely investigated in the combinations with antibiotics, ZnO and TiO_2 nanoparticles by preliminary results may interact with efflux pumps responsible for the resistance to many clinically important antibiotics, such as fluoroquinolones and more, which makes these combinations promising both in maintaining and increasing the activity of fluoroquinolones and other antibiotics and in reduction of their toxic effects.

Delivery of antibiotics via nanoparticles has advantages not only in increasing the concentration of the local antibiotic but also in minimizing the side effects that are associated with the use of broad-spectrum antibiotics, including those used to treat representatives of the normal microflora. Gold nanoparticles are perspective in delivery of antibiotics because in comparison with polymer-based nanoparticles, they are smaller, with a constant shape and size, are chemically stable and take part in multivalent interactions between the antibiotic and bacterial surface.

Considering perspectives for future studies, particularly over the next 5 years, they can be directed to solve several questions. Published studies have demonstrated the presence of different types of interactions between metallic nanoparticles and antibiotics from antagonistic to synergistic effects. Although some articles propose possible explanations for this synergistic effect, it is not yet fully understood. Researchers generally associate the enhancing effect with an impairing activity of antibiotics on cell walls and more significant accumulation of nanoparticles inside bacterial cells. It was also discussed that combined effects of antibiotics with nanoparticles were more pronounced on Gram-negative bacteria than Gram positives owing to the thinner Gram-negative cell walls. However, in some studies

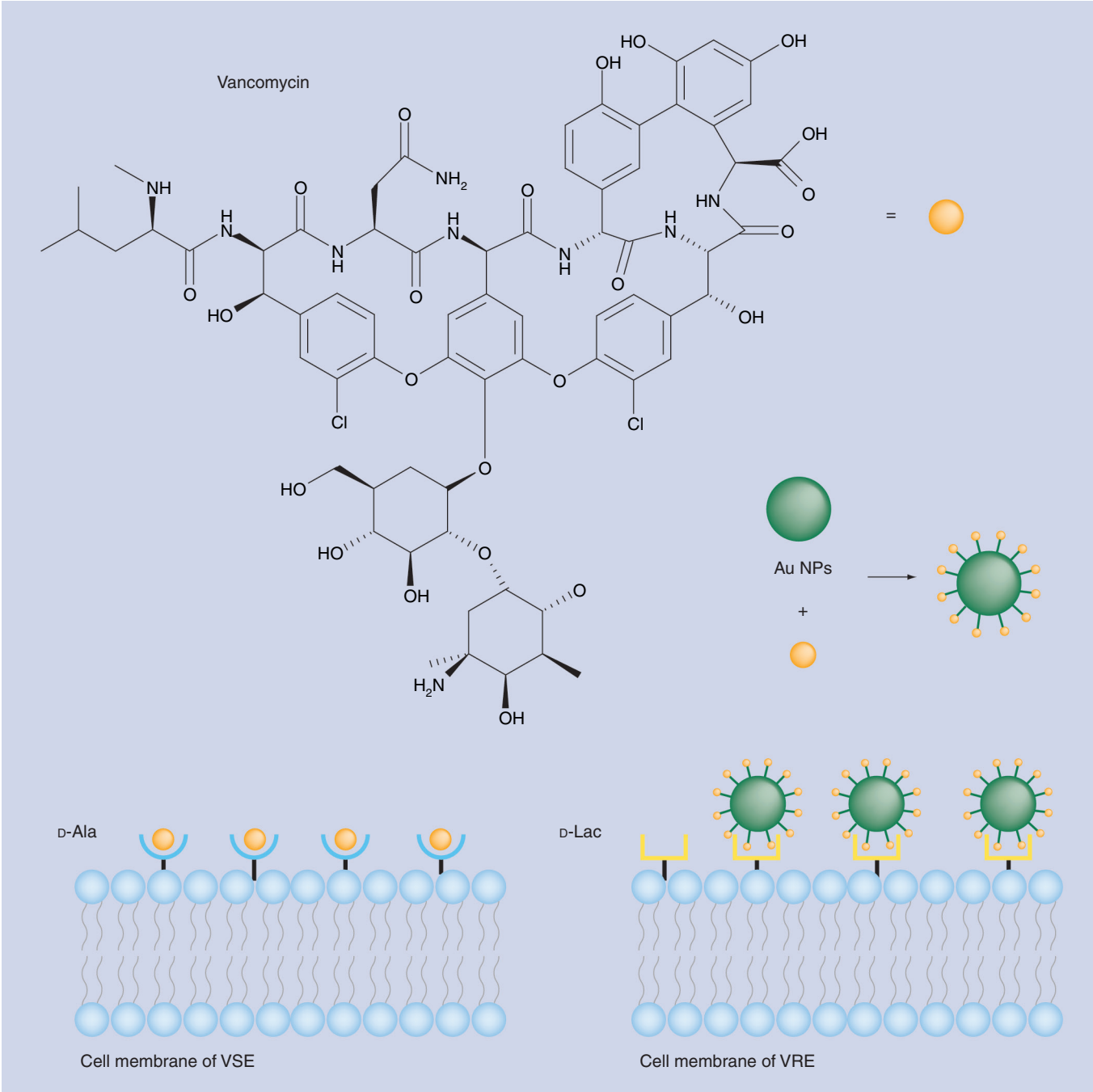

Figure 5. Mechanism of interaction between vancomycin-capped gold nanoparticles and vancomycin-resistant enterococci.
Vancomycin is unable to bind cell of vancomycin-resistant enterococci because of changes in its binding sites, such as terminal cell-surface peptides; vancomycin-capped gold nanoparticles bind amino acid residues in nonspecific multivalent way and thus, vancomycin restores its properties and can further inhibit cell wall synthesis.
Au: Gold; D-Ala: D-alanine; D-Lac: D-lactate; NP: Nanoparticle; VRE: Vancomycin-resistant enterococcus; VSE: Vancomycin-sensitive enterococcus.
Adapted from [38].

it was demonstrated that combination of nanoparticles with antibiotics that inhibit protein synthesis of bacteria had also high antibacterial efficacy. Therefore it is evident that there is an urgent need to research the mechanisms of action behind nanoparticle and antibiotic combinations to more clearly understand them.

It is believed that one of the important steps in combating antibiotic-resistant bacteria is impairing the structures of proteins that are responsible for resistance. Therefore, we think that studies must be focused on combinations of antibiotics with nanoparticles that strongly interact with proteins and impair their structures. By this method, resistance against antibiotics may be overcome

Table 2. Studies on antibiotic–nanoparticle combinations.

Nanoparticle size (nm)	Test object	Antibiotics in combination	Observed effect in the presence of nanoparticles	Mechanism of effect	Ref.
Silver					
20	*Escherichia coli*	Amoxicillin	Significant reduction of bacterial growth	• Different mechanisms of action of penicillins and nanosilver work in additive manner, for example, penicillins destroy bacterial cell wall and increase its penetration for silver nanoparticles • Bonding reaction between amoxicillin and silver nanoparticles leads to increased concentration of antimicrobial agents contacting with bacterium • Nanosilver acts as a antibiotic's carrier and facilitates approach of hydrophobic antibiotic to bacterial cell membrane • Silver chelates prevent DNA from unwinding, and when nanosilver's antimicrobial groups interact with the DNA, higher amount of bacteria hold back the unwinding of DNA. This results in more serious cell damage • Differences in activity of combinations against Gram-positive and Gram-negative bacteria may be explained by differences in cell wall structure and more difficult penetration of Gram-positive cell wall accordingly to the results of [27,30]. Ampicillin destroys cell wall and increases its penetration for silver nanoparticles	[34]
20	*Staphylococcus aureus*, *E. coli*	14 antibiotics from different groups	Enhancement of antibacterial action of penicillin G, amoxicillin, erythromycin, clindamycin and vancomycin against both tested strains with a more pronounced effect against *S. aureus*	• Same as for 20-nm nanoparticle using *E. coli* as test object	[29]
5–15	*S. aureus*, *E. coli*, *Micrococcus luteus*, *Salmonella typhi*	Ampicillin, erythromycin, kanamycin and chloramphenicol	Increase in activity was seen in all tested combinations, especially in amoxicillin. In general, an increase in activity was more prominent against Gram-negative bacteria.	• Same as for 20-nm nanoparticle using *E. coli* as test object	[27]
60–80	*S. aureus*, *E. coli*, *Pseudomonas aeruginosa*	Ampicillin, gentamicin, kanamycin, streptomycin and vancomycin	The highest enhancing effect was observed in vancomycin. In general, increase of activity was more prominent against Gram-negative bacteria.	• Same as for 20-nm nanoparticle using *E. coli* as test object	[30]

MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant enterococci; VRSA: Vancomycin-resistant *S. aureus*.

Table 2. Studies on antibiotic–nanoparticle combinations (cont.).

Nanoparticle size (nm)	Test object	Antibiotics in combination	Observed effect in the presence of nanoparticles	Mechanism of effect	Ref.
Silver (cont.)					
25	*E. coli, Acinetobacter calcoaceticus, Ecdyonurus helveticus, Aeromonas bestiarum, Proteus myxofaciens, Pseudomonas fluorescens, Bacillus subtilis, Kocuria rhizophila, Micrococcus luteus*	Antimicrobial peptides (polymyxin B, gramicidin A, alamethicin, gramicidin S, PGLA and magainin 2)	Synergy with polymyxin B and gramicidin S, other combinations were additive	• Polymyxin B increases permeabilization of the outer membrane of the bacterial cell and enhances the intrinsic antibacterial effect of silver nanoparticles	[36]
Titanium dioxide					
20	MRSA	23 antibiotics from different groups	Enhancement of antibacterial activity of most antibiotics, especially of penicillin, amikacin, ampicillin, gentamycin, oxacillin and cloxacillin	• Possible mechanism of enhancement of penicillin activity may be similar to silver nanoparticles, such as increasing the cell membrane permeability for nanoparticles • Nanoparticles may also inhibit efflux of antibiotics from the bacterial cell	[31]
Zinc oxide					
80	*S. aureus*	25 antibiotics from different groups	Enhancement of antibacterial activity of most antibiotics, especially of penicillin, amoxicillin/clavulanic acid, amikacin, methicillin, oxacillin, ampicillin, amoxicillin, gentamicin and cloxacillin	• Possible mechanism of enhancement of penicillin activity may be similar to silver nanoparticles, such as increasing the cell membrane permeability for nanoparticles • Nanoparticles also may inhibit efflux of antibiotics from the bacterial cell	[32]
20–45	*S. aureus*, *E. coli*	15 antibiotics from different groups	Decreasing activity of most antibiotics in combination. Concentration-dependent increasing activity of ciprofloxacin	• Increased activity of ciprofloxacin in presence of ZnO nanoparticles can be attributed to inhibition of ciprofloxacin efflux from the cell due to inteference of ZnO nanoparticles with pumping NorA protein, activation of ciprofloxacin uptake by influencing activity of membrane Omf protein, and by binding reaction between ciprofloxacin and ZnO nanoparticles stabilizing the ciprofloxacin–ZnO nanoparticle complex	[28]
2	MRSA, *E. coli*	Methylene blue embedded into polysiloxane polymer	Increase of photodependent oxidative action of methylene blue	• Gold nanoparticles enhance the light-induced antimicrobial activity of methylene blue by possible increase of the yield of reactive oxygen species rather then singlet oxygen	[37]

MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant enterococci; VRSA: Vancomycin-resistant *S. aureus*.

Table 2. Studies on antibiotic–nanoparticle combinations (cont.).

Nanoparticle size (nm)	Test object	Antibiotics in combination	Observed effect in the presence of nanoparticles	Mechanism of effect	Ref.
Gold					
3	*E. coli*, *P. aeruginosa*	Amino-substitued pyrimidines	Pyrimidines exhibit bacterial action despite being nonactive themselves	• Pyrimidine-capped gold nanoparticles cause sequestration of magnesium or calcium ions, and this leads to disruption of the bacterial cell membrane, and leakage of cellular content including nucleic acids; they also interact with DNA and inhibit protein synthesis	[35]
15	*E. coli*	Gentamicin	No influence on activity of gentamicin	Nonstability of antibiotic–gold nanoparticle suspension leads to aggregation of gold nanoparticles after addition of antibiotics and inhibits cooperation between them. Therefore, preparation of stable conjugates and a large enough amount of antibiotic is necessary to achieve cooperation between gold nanoparticles and antibiotics. Under these conditions gold nanoparticles may act as antibiotic carriers	[33]
4–5	VRE, *E. coli*	Vancomycin	Enhancement of vancomycin activity against VRE and appearance of activity against *E. coli*	• In VRE, binding sites for vancomycin are changed, thus contributing to its resistance. Gold nanoparticles coated with vancomycin multivalently interact with bacterial surface receptors allowing the binding of the vancomycin molecule to the bacterium, thus increasing its activity	[66]
4–5	*E. coli*, *S. aureus*, VRSA	Vancomycin	Enhancement of vancomycin activity against VRSA and appearance of activity against *E. coli*	• Nonspecific binding of nanoparticles to cell-surface peptides on VRSA that are involved in the synthesis of the cell wall	[71]

MRSA: Methicillin-resistant *Staphylococcus aureus*; VRE: Vancomycin-resistant enterococci; VRSA: Vancomycin-resistant *S. aureus*.

and antibacterial efficacies of combinations can be enhanced.

Taking into account photodependent activity of some nanoparticles, for example TiO_2, studies of interactions between antibiotics and these nanoparticles under different conditions of light irradiation should also be performed.

We also think that properties of nanoparticles such as size, shape and stability can affect their interactions with antibiotics. Furthermore, since bacteria were demonstrated to have nanosize metals inside their cells [71], we also think that the types and amounts of these metals may be very important for further applications of antibiotics. Presence of nanoparticles inside the bacterial cell should be determined, and we propose that antibiotics should be used taking into consideration their interactions with intracellular nanoparticles. Antibiotics that demonstrate high antibacterial activity with nanoparticles during *in vitro* studies should be used. Furthermore, interactions of antibiotics with intracellular nanoparticles allow the use of nanoparticles at very low dosages and prevent their toxic effects on humans. However, we believe that this kind of application needs more investigation.

Conversely, researchers studying interactions between metallic nanoparticles and antibacterial agents are mainly devoted to conventional antibiotics. However, combinations of nanoparticles with antimicrobial peptides, antiseptics and especially with natural antimicrobial agents such as plant essential oils require greater attention and these studies can be done in the next 5 years.

In summary, we think that these reviewed studies represent a new and promising approach for overcoming drug resistance problems all over the world. Results of the studies generally indicate that combinations of metallic nanoparticles with antimicrobial agents have significant potential to cope with drug resistance in bacteria. We believe that the resistance problem may be solved by making more investigations related to this subject.

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Key issues

- The progressive increase in the number of multidrug-resistant bacteria documented throughout the world makes it necessary to search for new methods for coping with microbial resistance.
- Metallic nanoparticles with antibacterial properties represent an alternative method for coping with antibiotic resistance, and combinations of these nanoparticles with antibiotics provides an opportunity not only to increase antibacterial activity of both components but also to reduce their toxic effects.
- Methods of assessment of interactions between nanoparticles and antibiotics are not well standardized which makes it more difficult to compare results of studies. The most commonly used method is disk diffusion while more informative methods, such as checkerboard and time–kill curves, are very limited and merit more attention.
- The best studied and promising combinations are of silver nanoparticles with β-lactam and glycopeptide antibiotics; however, perspective interactions *in vitro* are also documented between titanium dioxide and different antibiotics, and between zinc oxide and fluoroquinolones.
- Widespread antibiotic resistance renewed interest in antimicrobial properties of natural antimicrobial compounds such as plant essential oils, and their combinations with nanoparticles also represents a promising approach to coping with microbial resistance.
- Biologically inert gold nanoparticles have demonstrated very promising ability to enhance antibacterial properties of vancomycin against multidrug-resistant bacteria, such as vancomycin-resistant enterococci and vancomycin-resistant *Staphylococcus aureus*. The toxicity and clinical efficacy of these combinations should be evaluated in further detail.

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