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Нанотехнологии в свете современных антибактериальных стратегий (обзор)

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Резюме: *Введение*. Появление и рост бактериальных штаммов с множественной лекарственной устойчивостью в последние десятилетия связаны с широким и бесконтрольным использованием антибиотиков, а также снижением количества результативных исследований и открытий новых классов антибактериальных препаратов. Эти тревожные тенденции признаются одной из серьезных угроз для глобального общественного здравоохранения. Они стимулируют и повышают актуальность масштабного поиска и изучения новых антимикробных стратегий, альтернативных традиционной антибиотикотерапии. *Целью* обзора является критический разбор преимуществ и ограничений современных антимикробных платформ с акцентом на инновационных технологиях использования наночастиц для прямого или опосредованного воздействия на патогенные бактерии, в том числе тех, которые обладают мультиустойчивостью к традиционным антибиотическим препаратам. *Материалы и методы*. Поиск источников проводился в ресурсах Кокрановской библиотеки (директория Wiley Online Library), EMBASE (EMBASE.com), CINAHL, Web of Science. Глубина поиска – 2017–2021 гг. *Результаты исследования*. Значительная часть положительных терапевтических эффектов для диагностики и лечения инфекций была получена в результате реализации принципиально новых механизмов антимикробного действия наноразмерных частиц и других наноматериалов. Оценивая будущие перспективы нанотехнологий в качестве наиболее динамично и активно развивающейся в последние годы антимикробной стратегии, следует сделать вывод, что эти инновационные платформы, безусловно, заслуживают пристального внимания и дальнейшего изучения в качестве альтернативного средства профилактики и лечения бактериальных инфекций. Основным ограничением для клинического использования современных наноматериалов является необходимость дальнейшей оценки их безопасности и цитотоксичности. *Заключение.* Борьба с устойчивостью к антибиотикам требует совместных действий общественных и государственных институтов. Разработка безопасных и эффективных антибактериальных технологий должна сочетаться с принятием международной программы жесткого регламентирования и строгих мер контроля за обоснованностью и рациональным использованием антибиотиков и других антибактериальных препаратов в медицине, косметологии, сельскохозяйственном производстве.

Ключевые слова: бактерии, множественная лекарственная устойчивость (МЛУ), антибактериальные стратегии, нанотехнологии, наночастицы.

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Nanotechnologies in the Light of Modern Antibacterial Strategies: A Review

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Summary. *Introduction*: The emergence and growth of multidrug-resistant (MDR) bacterial strains in recent decades is associated with the widespread and uncontrolled use of antibiotics, as well as a decrease in the number of effective studies and discoveries of new classes of antibacterial drugs. These alarming trends are recognized as a major threat to global public health. They stimulate and increase the relevance of a large-scale search and study of new antimicrobial strategies, alternative to traditional antibiotic therapy. *The purpose* of the review is a critical analysis of advantages and limitations of modern antimicrobial platforms with an emphasis on innovative techniques of using nanoparticles for a direct or indirect effect on pathogenic bacteria, including the MDR ones. *Materials and methods*: The search for literary sources published in 2017–2021 was carried out in the resources of the Cochrane Library (Wiley Online Library directory), EMBASE (EMBASE.com), CINAHL, and Web of Science. *Results*: Most positive therapeutic effects for the diagnosis and treatment of infectious diseases were obtained by implementing fundamentally new mechanisms of antimicrobial activity of nanosized particles and other nanomaterials. When assessing future prospects of nanotechnology as the most dynamically and actively developing and promising recent antimicrobial strategy, it should be concluded that these innovative platforms certainly merit attention and further study as alternative means of preventing and treating bacterial infections. The main limitation for the clinical use of modern nanomaterials is the need for further assessment of their safety and cytotoxicity. *Conclusions*: Tackling antibiotic resistance requires the concerted action of community and government institutions. The development of safe and effective antibacterial technologies should be accompanied by adoption of an international program of strict regulation and tough measures of control over validity and rational use of antibiotics and other antibacterial drugs in medicine, cosmetology, and agriculture.

Keywords: bacteria, multidrug resistance (MDR), antibacterial strategies, nanotechnology, nanoparticles.

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Background. The emergence of multidrug-resistant (MDR) bacterial strains in recent decades has been mainly related to a widespread and uncontrolled use of antibiotics and the lack of production of new antibacterial drugs. The rapid dissemination of strains of pathogenic and opportunistic MDR bacteria is recognized by the World Health Organization (WHO) as one of the most severe threats to global public health [1].

Today, antibiotic-resistant bacteria kill about 700,000 people annually. According to WHO experts, if measures are not taken in the coming years, by 2050, the number of deaths from bacterial infections worldwide may exceed the number of deaths from cancer and rise to 10 million a year. Up to $ 100 trillion will be required to treat patients with bacterial infections [1, 2]. This serious socio-economic problem increases the urgency of a large-scale search and study of new antimicrobial strategies that could satisfy the urgent need to treat drug-resistant bacterial infections [2, 3].

Antibiotics are currently used as the primary antibacterial strategy for treating bacterial infections, and about 50 new drugs are now in clinical trials [1, 3]. However, many of these dosage forms are synthetic analogs of the known classes of natural antibiotics [2, 4].

In fact, the development and introduction of novel antibacterial drugs is a strictly regulated time- and resource-consuming process. Unfortunately, an alarming trend towards a sharp decline in the number of effective studies and discoveries of new classes of antibiotics that are active against priority pathogens has been observed recently. In this regard, the need for new and effective antimicrobial strategies, alternative to traditional antibiotic therapy, has become even more relevant [2, 4, 5].

In February 2017, WHO compiled a list of high-priority antibiotic-resistant pathogens for which the need to develop new antimicrobial agents was identified as "urgent" at the global level [1]. It includes bacteria of the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*), gram-negative MDR bacteria of the *Enterobacteriaceae* family, *Mycobacterium tuberculosis*, and *Clostridium difficile* [4–6]. Many countries witness a significant increase in the rates of diseases caused by methicillin-resistant strains of *S. aureus* (MRSA), and infections associated with this pathogen are recognized as one of the most common causes of death worldwide [1, 7].

Due to the lack or a limited number of therapeutic agents for treating the diseases induced by these bacteria, including pneumonia, urinary tract infections, wound infections, and sepsis, the need to develop new antimicrobial approaches is critical [2, 8]. To this end, several strategies have been proposed recently, which formed the basis for creating several therapeutic drugs. The most promising of them are at different stages of experimental and clinical trials to assess their practical efficacy, safety, drug compatibility, and the absence of side effects [3, 7].

The proposed innovative antimicrobial strategies, which have attracted the attention of experts and clinicians over the past 10–15 years, may be conditionally divided into: a) approaches aimed directly at bacteria, and b) methods that modulate the immune response or inhibit the virulence mechanisms of bacteria with fundamentally new principles of action [2, 5, 8] (Fig. 1).

One of the most promising innovative directions in antimicrobial strategies is associated with nanotechnologies, novel and actively developing scientific areas. These modern technologies provide for the creation and use of nanomaterials and systems, the functioning of which is mediated by the structure of nanoparticles ranging in size from one to 100 nm. Among the promising antimicrobial strategies, application of nanoparticles is distinguished by pronounced antibacterial effects with the possibility of their potential benefit to combat infectious agents and biological pollutants [9].

The **purpose** of this review is to give a critical analysis of advantages and limitations of modern

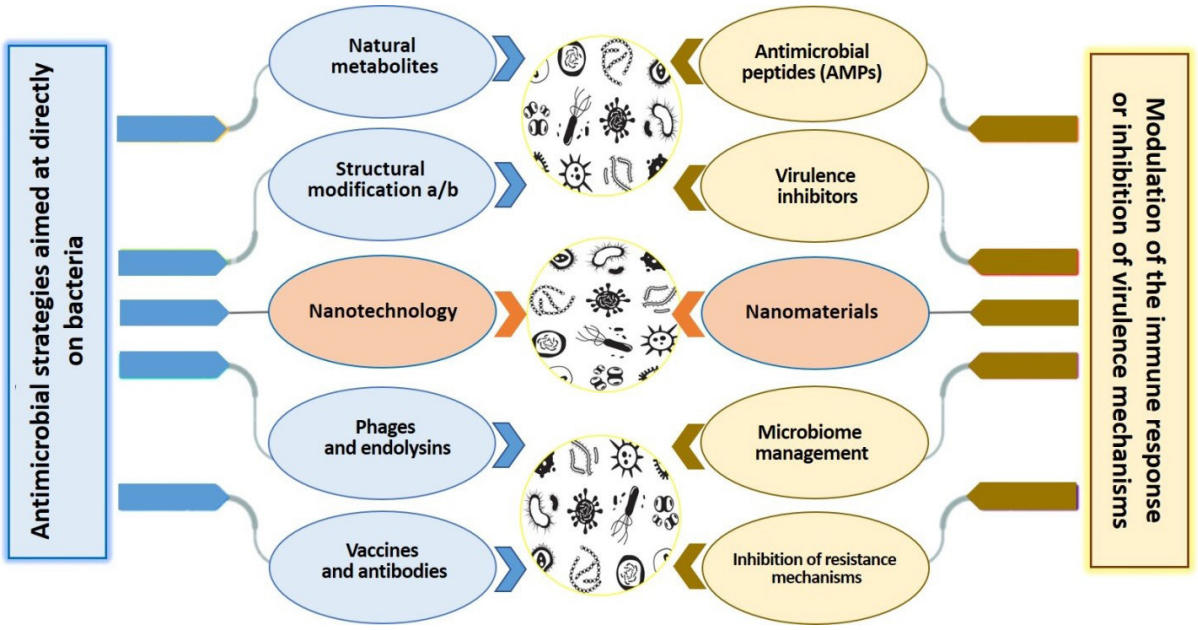

Рис. 1. Современные антибактериальные стратегии – потенциальная альтернатива традиционной антибиотикотерапии
Fig. 1. Modern antibacterial strategies: a potential alternative to traditional antibiotic therapy

antimicrobial platforms with an emphasis on innovative techniques of using nanoparticles for a direct or indirect effect on pathogenic bacteria, including those resistant to multiple traditional antibiotic drugs.

Materials and Methods

The search for literary sources published in 2017–2021 was carried out in the resources of the Cochrane Library (Wiley Online Library directory), EMBASE (EMBASE.com), CINAHL, and Web of Science.

Results

Origins and evolution of bacterial antibiotic resistance. The discovery and use of antibiotics in the 20th century revolutionized medicine and led to a change in the therapeutic paradigm that saved millions of human lives. The emergence of the antibiotic era became one of the most important public health events in the history of humankind while a succession of discoveries of new antibiotics in the 1960s and 70s instilled confidence in the quick victory over bacterial infections. Yet, the widespread and successful use of antibiotics gave rise to a rapid emergence of resistant strains of pathogenic bacteria [10, 11].

Studying the nature of microbial drug resistance led to the discovery of its genetic mechanisms, which was of decisive importance in developing key directions in the search for new antimicrobial strategies [10]. Today, the bio-information database includes over 20,000 genetic elements, site-specific recombination integrons, mediating antibiotic resistance in almost 300 species of microorganisms [3, 4, 10].

On the one hand, this proves that bacterial resistance to antimicrobial drugs is an ancient and natural process. On the other hand, it raises concerns about the future of the humanity due to the onset of the post-antibiotic era, which approach is accelerated by the uncontrolled, excessive, and unreasonable use of antibiotics for numerous purposes in medicine, agriculture, trade, cosmetology, and everyday life that forms the "acquired" resistance of microorganisms [2, 8]. Nowadays, natural ecosystems are saturated with these substances, contributing to the emergence and selection of antibiotic-resistant bacterial strains, the development and distribution of which in the populations result from human activities [4, 11, 12].

Blair et al. [12] distinguish two main types of bacterial antibiotic resistance. Type I is mediated by the genetic determinants of microorganisms (intrinsic resistance). Type II is associated with biochemical mechanisms of defense, evasion, or destruction of antibiotics (evolutionary resistance) [12].

Bacteria have exceptional genetic plasticity, allowing them to respond to a wide range of environmental stresses, including the presence of antimicrobial molecules that threaten their survival. Being in the same niche with other microorganisms producing antimicrobial substances, bacteria have developed ancient mechanisms that allow them to resist and maintain viability [4, 11]. From an evolutionary point of view, bacteria use two main genetic strategies to adapt to antibiotics: i) formation of resistant dormant cell forms through the activation of toxin-antitoxin systems [10, 13], and ii) acquisition of foreign DNA encoding the determinants of resistance through horizontal gene transfer, which is one of the most important factors in the evolution of bacteria and a common cause of the development of antimicrobial resistance [10, 11].

Over millions of years of evolution, bacteria have developed complex biochemical mechanisms of resistance to antimicrobial substances to escape death. At the same time, resistance to one class of antibiotics is usually is achieved by several biochemical pathways. Besides, one species of bacteria can use various resistance mechanisms, such as the production of β-lactamases or other enzymes to inactivate, destroy or modify the chemical structure of antimicrobial compounds, decrease the membrane permeability, genetic mutations, activation of efflux pumps that remove the antibiotic from the cell, and protection (modification) of target sites [4, 10]. Each biochemical mechanism is encoded by specific genetic determinants of microorganisms, thus mediating intrinsic resistance to antimicrobial drugs or toxic compounds.

For example, genes encoding β-lactamases (bla) found on the chromosome were located in mobile genetic elements, as part of an additional genome or elements of integrons [3, 7].

The consequence of the uncontrolled bacterial growth is a high prevalence of biofilms, i.e. communities of microorganisms usually consisting of several species and covered with a self-reproducing protective extracellular matrix, which makes bacteria resistant to antimicrobial agents and the immune system and causes chronic infections [7, 11].

A thorough understanding of the mechanisms by which bacteria become resistant to antibiotics is of paramount importance for developing new antimicrobial strategies and developing new therapeutic agents. However, despite the tempting prospects that open up, it should be understood that there is a great distance from discovering a new antibiotic agent from natural products to its use in the clinic. Making a new pharmaceutical remains challenging and often economically unjustified. It is sometimes difficult to produce active metabolites of natural products in the required quantities, and their antimicrobial activity in vivo depends on many factors associated with technological methods of isolation and purification, method of application, method of administration, etc. [8, 10–12]. The hopes of humankind for the future are therefore increasingly associated with antimicrobial strategies that may become real alternatives to traditional antibiotic therapy in the future.

Promising Antimicrobial Bacteria-Targeted Strategies

Natural products with new mechanisms of antimicrobial action. Most existing classes of antibiotics come from natural sources with the exception of those created by chemical synthesis. Metabolites of terrestrial and marine organisms, plants, and microorganisms remain a promising source of new drugs with antimicrobial action. The targets of their chemical components include bacteria (compounds with bacteriostatic or bactericidal activity) and critical factors of pathogenicity (antiadhesive, antioxidant, antibiofilm, and other activities). Both traditional and new approaches are used to isolate biological activity components and produce fascinating findings [8, 11, 14, 15].

A recent example of a new antibiotic is teixobactin [16], a macrocyclic depsipeptide natural product isolated from uncultivated bacteria (dormant cell forms) *Eleftheria terrae*, which are members of

complex soil microbial communities. The structure of teixobactin contains a rare amino acid (L-allo-enduracidine), which plays a key role in providing high antibacterial activity against several gram-positive pathogenic bacteria and *M. tuberculosis* by inhibiting cell wall synthesis. Yet, the same amino acid is also the main limiting factor in developing synthetic analogs of the new antibiotic, which prevents the complete synthesis of teixobactin, making it laborious and inefficient [16, 17].

A new approach based on metagenomics, a powerful analytical tool independent of cultural methods, has opened access to collective genomes of bacterial populations of various natural ecosystems or, to be more exact, to microbial clusters of biosynthetic genes, i.e. organized groups of genes involved in the production of specialized metabolites with antibiotic activity, most of which is not expressed in laboratory conditions [18]. Bacteria use these metabolites (e.g., ferroverdins and bagremycins) as weapons in interspecies competitive interactions.

Structural modifications of existing classes of antibiotics. In the search for new antimicrobial agents, structural analogs of available antibiotics of various classes have been developed in recent years, which increases and expands the spectrum of their antimicrobial activity and may in the long term reduce toxicity of intestinal bacteria or their commensal microbiota [7, 11]. This approach, combined with the creation of hybrid (heterodimeric) structures based on the covalent connection of antibacterial drugs (or their pharmacophores) of various classes, is a promising modern strategy for overcoming bacterial resistance [11, 12]. Antibiotic hybrids provide previously unavailable compounds that can be used as separate antibacterial agents or as adjuvants that enhance the primary antibiotic(s) activity.

A recent study by Okano et al. [19] presented the design of such a hybrid based on glycopeptide antibiotics, including vancomycin, with three independent mechanisms of antimicrobial action targeting vancomycin-resistant enterococci (VRE). The new hybrid antibiotic destroys the molecular basis for the formation of resistance to vancomycin and has a 200-fold higher antimicrobial activity against VRE. Besides, its additional structural modifications mediate the emergence of two other independent antibacterial action mechanisms that were not found in the original antibiotics [19].

Several such hybrid antibiotics are currently undergoing phase III clinical trials, including cadazolid, which has strong lipophilic properties and a powerful antimicrobial effect against *C. difficile*, a gram-positive spore-forming anaerobe, the priority etiological agent of nosocomial diarrhea in the world [10, 20, 21]. At the same time, the analysis of the results of comparative studies of the efficacy of this antibiotic with vancomycin did not reveal any advantages, which requires additional research [21].

Phage therapy and endolysins. Another promising strategy for combating MDR infections is the use of lytic bacteriophages to treat bacterial infections. Bacteriophages are bacteria viruses that can cause lytic or lysogenic infections in bacteria after attaching and incorporating their genome into bacteria. Phage proteins and replicated genomes are synthesized and self-assembled into new viral particles during lytic infection, which ultimately lyse the bacterium [22, 23].

The antimicrobial activity of phages has been known for a long time. Already in 1896, the British bacteriologist Ernest Hankin noted the activity of river water against *Vibrio cholerae* and suggested a presence of a filtering substance, which might have limited the cholera epidemic in India. A similar phenomenon was later noted by the Russian microbiologist Nikolay Gamaley in relation to *Bacillus subtilis* [cit. by 22] and laid the basis of the research into this phenomenon, isolation of non-bacterial microorganisms, description of their properties, and the use of phage therapy for bacterial infections, after which patients recovered within one or two days [cit. by 23].

Subsequent studies have demonstrated that a specific property of phages is selectivity of their action and the key stage in phage therapy protocols is the specific selection and isolation of phages. This is why a widespread practice in phage therapy is the use of phage cocktails (Pyophage and Intestiphage) having a broad spectrum of action [22, 23].

Traditionally, this antibacterial strategy is actively studied and used in Georgia and Poland. However, due to the increasing antibiotic resistance of bacteria around the world, the interest in this antimicrobial activity of phages has increased in the countries of Southeast Asia and the United States [5, 22–24]. Both academic institutions and the pharmaceutical industry in many countries recognize the importance of phage therapy for bacterial infections.

High efficiency, safety for eukaryotic cells, the absence of toxic effect, a long-term experience in studying phages and using phage therapy still do not outweigh the main limitations of their use in clinical practice. The latter are associated with the difficulty of standardizing treatment due to differences in biological, physical, and pharmacological properties of bacteriophages compared to conventional antimicrobial drugs [5, 23]. These obstacles impede issuing permits for phage therapy while all pre- and clinical trials get limited to studies of safety and efficacy of local phage treatment or their combined use with traditional antibiotics [8, 22, 24].

When studying bacteriophages, enzymes (endolysins and peptidoglycan hydrolases) were isolated that destroy the cell wall of target bacteria and represent an interesting alternative to conventional antibiotics [24, 25]. Endolysins obtained from bacteriophages are necessary to destroy the cell wall of target bacteria and are a promising alternative to antibiotics as therapeutic lysines that kill certain bacteria while preserving the microbiota [5, 23, 24]. Molecular engineering of endolysins once used to be applied to the development and creation of new antimicrobial drugs [23–25].

The advantages of endolysins, compared to phage therapy, are associated with the possibility of expanding the lytic spectrum by replacing or adding specific domains outside the serovar or target bacterial species. Besides, endolysins can also act synergistically in combination with other phage lytic enzymes or antibacterial agents, including antibiotics [22, 24, 25].

Compared to traditional antibiotics, endolysins lyse target bacteria faster, show high efficacy against MDR gram-positive bacteria and the ability to act in biofilms, including on the surface of mucous membranes [23–25]. Unlike intact phages and

antibiotics, bacteriophage endolysins have a unique property: they bind and destroy highly conserved peptidoglycan structures inside the cell wall, thus preventing the development of resistance [24, 25].

Some endolysin-based drugs are currently undergoing phase II and III clinical trials. The first therapeutic agent SAL200 against methicillin-resistant S. aureus (MRSA) strains for intravenous infusion was recently obtained from the group of endolysins [25]. Yet, when analyzing the effectiveness of drugs of this type, cases of allergic reactions and a complete lack of activity against gram-negative bacteria were found [24, 25].

Vaccines and monoclonal antibodies. In the context of a decrease in the production of new antibiotics, vaccination is considered as one of the antimicrobial strategies and first lines of defense against MDR bacterial pathogens, which may prevent infection and make treatment unnecessary. The main targets of the developed vaccines and the antibodies produced are bacterial receptor proteins, which are essential components of cell adhesion or signal transduction [26, 27].

Ginsburg and Klugman [27] suggest that in many countries the reduction in the number of MDR pathogens could be more easily achieved using vaccines rather than traditional interventions, including the improvement of hygiene and sanitation [27]. The experience of using pneumococcal conjugate vaccines against *Streptococcus pneumoniae*, with a high incidence of antibiotic resistance, convinces of the need to consider the impact of vaccination as an essential tool in combating bacterial resistance to antimicrobial drugs [26].

Vaccines do reduce the prevalence of resistance by decreasing the need for antimicrobial drugs and the overall disease incidence rate. However, the development of new and effective vaccines is impossible without studying the immune mechanisms of defense. Besides, the development of vaccines against bacterial pathogens requires a deep understanding of how vaccination affects the growth and spread of the bacteria in the human body. The availability of this critical information must be considered when evaluating the efficacy of a vaccine [26, 27].

Combination of targets when using multivalent vaccines is a promising trend in this antimicrobial strategy. For example, the induction of high titers of antigen-specific antibodies resulting from active immunization with multivalent vaccines against *S. aureus* antigens is an encouraging result [7, 27]. However, the question of whether these antibodies play a decisive role in human protection requires further study. The history of antibacterial vaccines remembers examples of negative results of clinical trials and even doubts about their safety [7, 26, 28].

The prophylactic or therapeutic use of specific monoclonal antibodies (mAbs) to prevent or treat bacterial infections is also considered to be one of the promising antimicrobial strategies [29–31]. Results of preclinical trials prove that mAbs can also effectively act synergistically with antibiotics. Being targeted and highly specific methods of treatment, they are enable to induce bacterial drug resistance or affect the commensal flora of the normal gut microbiome [29, 31].

The most common bacterial targets of mAbs are surface antigens and the main mechanisms of their action include inhibition of virulence factors, complement-mediated lysis of gram-negative bacteria, and neutralization of toxins from gram-positive pathogens (e.g., *Bacillus anthracis*, *C. difficile*) [7, 29, 30]. However, in practice, many of the encouraging results obtained in vitro have failed to be confirmed in clinical trials. For example, Vuong et al. [7] reported negative results of *in vivo* approbation of pagibaximab, a mAb developed against *S. aureus* lipoteichoic acids [7].

An example of a few successful and most notable achievement in the clinical use of mAbs is bezlotoxumab, an antibody-based drug that targets *C. difficile* toxins. This safe and well-tolerated preparation with a low risk of severe side effects is registered in the United States as an adjunctive therapy to prevent recurrent infections associated with *C. difficile* [32]. However, in practice, the effectiveness of this and similar toxin-specific mAbs is limited to a relatively narrow range of bacterial agents and depends on the multifactorial nature of the pathogenetic mechanisms of infections, including those associated with toxins [29, 30, 32]. Apart from that, a severe limitation of the potential use of mAbs is their high cost, which may restrict their use as an alternative treatment in low- and middle-income countries [29, 31, 32].

Promising antimicrobial strategies aimed at modulating the immune response or inhibiting bacterial virulence mechanisms. Antimicrobial peptides (AMPs). The status of the host's immune system is an essential but often neglected factor in preventing and treating drug-resistant infections. Pathogenic microorganisms actively suppress immune responses of the host by releasing specific mediators and regulators, which, in their turn, become factors of pathogenicity that induce the development of the disease. The purpose of this antimicrobial strategy is to stimulate and enhance protective antimicrobial immunity while protecting against tissue damage caused by inflammation. This direction provides for the active use of new and non-traditional anti-infective drugs aimed at the receptors of innate immunity of peptides – regulators of innate defense (antimicrobial peptides (AMPs). Several immunomodulators have been developed based on AMPs to increase the efficacy of antimicrobial therapy by enhancing both innate and adaptive immune responses in an infected organism (so-called immunologic adjuvant) [33–36].

Natural AMPs are evolutionarily conserved structurally and functionally diverse protein molecules present in almost all living organisms. For example, peptides are the essential components of innate immunity in humans and other higher organisms, providing the first line of defense against infections [33, 35]. As of the beginning of 2021, the international database contains over 3,500 such peptides [34]. Despite co-evolution with bacteria, AMPs have retained their antimicrobial activity while bacteria have not yet developed widespread resistance to them.

Most AMPs kill microbial pathogens directly, while many of them have a broad spectrum of antimicrobial activity, including that against gram-positive and gram-negative microbes [33, 36]. Thus, AMPs have many attractive features of the new class of antibiotics, such as a broad spectrum of activity, a low frequency of bacterial resistance to them, and a special mode of action that involves the formation of pores in the cytoplasmic membrane.

Their amino acid sequences, positive charge, and very small size allow peptides to bind to microbial membranes and destroy them [33, 34]. Other studies have shown that AMPs can also inhibit biosynthesis of the cell wall, nucleic acids, and proteins [36]. Therefore, interest in the therapeutic use of these molecules is constantly growing.

Several AMPs have been proposed as a potential basis for creating new generation antibiotics, which are currently being evaluated in the later stages of clinical trials not only as anti-infectious drugs but also as innovative candidate products for immunomodulation accelerating wound healing and preventing postoperative scars [34]. A strong synergistic activity of these peptide molecules with clinically used antibiotics, such as vancomycin, penicillin, ampicillin, azithromycin, ciprofloxacin, etc., was established [33–35]. Several well-characterized cyclic anti-infective peptides are already in clinical use (gramicidins and polymyxins).

However, despite generally favorable reviews of the effectiveness and safety of AMPs, their tropism to the membranes of some eukaryotic cells (e.g., erythrocytes) leading to destruction and hemolysis of the latter was noted [34]. Consequently, most of the peptides undergoing preclinical and clinical trials today have been developed for local applications such as acne and wound healing.

Approaches inhibiting bacterial virulence mechanisms. Neutralization of pathogenicity factors prevents pathogens from using their virulence factors during infection [4, 7]. Targeting virulence factors such as bacterial adhesion or biofilm formation may lead to new anti-infectious therapies. In this regard, innovative antibiofilm agents with new targets and modes of action deserve attention. It is known that over 80 % of microbial infections are associated with biofilms and that the growth of microorganisms in biofilms can increase their resistance to antimicrobial agents. However, antimicrobial therapy is often powerless against pathogenic microorganisms embedded in the matrix of extracellular polymeric substances [7, 33, 35].

Among the innovative antimicrobial approaches, researchers yet again drew attention to AMPs and their properties associated with inhibiting the ability of bacteria to form biofilms [36]. For example, the synthetic peptide NA-CATH: ATRA1-ATRA1 and the natural AMP protein LL-37 from the cathelicidin family successfully suppressing the formation of *S. aureus* biofilms have been used for this purpose for almost 10 years now [37]. Such peptides as melamine, citropine, and lactoferrin have shown good anti-biofilm activity when infecting medical devices with *S. aureus* and *P. aeruginosa*, especially when administered together with rifampicin and minocycline [37, 38].

Another antibacterial strategy associated with the previous one mediates inhibition of one of the critical mechanisms of virulence – the adhesion of bacteria to receptors of eukaryotic target cells. Adhesion is an early and essential step in bacterial colonization of hard surfaces, which leads to biofilm formation and is a major cause of nosocomial infections. Modern possibilities of antiadhesive therapy prevent bacteria from realizing one of their crucial virulence mechanisms while making them more susceptible to antimicrobial therapy [33, 37].

A promising broad-spectrum bacterial target for antiadhesive therapy is poly-N-acetylglucosamine (PNAG), a conservative surface polysaccharide that is produced by almost all bacterial pathogens and is the main component of the extracellular matrix of *Staphylococcus* spp. biofilms [37, 38]. It has been found that antibodies that bind to PNAG and its deacetylated form are promising antibacterial agents *in vitro* and *in vivo* for a wide range of microorganisms. PNAG-based immunotherapy and human vaccines such as mAb F598 have been successfully tested in phase I clinical trials [38].

Human microbiota management. Microbiota disruption may have serious detrimental effects on human health. The human gut microbiota contains about 100 trillion microbial cells and affects general human physiology, especially metabolism, nutrient absorption, and maintenance of the normal brain and immune function [39]. The human microbiome is excessively exposed to antibiotics, which can lead to profound and long-term health consequences. In this regard, the relevance of developing and using antibacterial agents of a narrow spectrum of action, targeted only at pathogenic microorganisms with minimal harmful effects on the human microbiota, increases [6, 39].

Application of research approaches to the study of human microbiota has shown that the qualitative composition of these complex microbial communities is primarily mediated by the interspecies interaction of bacteria, which can be cooperative or, more often, competitive. Stubbendieck and Straight [40] describe two modes of microbial competition: interference and operational. Interference competition is carried out through the secretion of specialized metabolites by microorganisms, many of which have a broad spectrum of antimicrobial activity. The study of these metabolites has led to the discovery of penicillin and cephalosporin. Other metabolites have a narrow range of activity and include bacteriocins and AMPs, which target closely related organisms [34, 40]. Examples of the mechanisms of this type of competition are contact-dependent inhibition systems (CDI), type 6 secretion (T6SS), and quorum sensing suppression signaling molecules [40].

Modern approaches to intestinal microbiota management are associated with the active use of probiotics, prebiotics, or their combinations called synbiotics [4, 7, 40]. Most probiotics are obtained from lactic acid bacteria, and their effect on the digestive flora depends on the bacterial strain and is determined by the production of bacteriocins [38, 39]. Probiotics produce a variety of antimicrobial metabolites that are used competitively by gut bacteria that can inhibit or kill other gut microbes and pathogenic bacteria. It was found that some bacterial metabolites have a powerful antibacterial effect on pathogenic flora without a negative impact on human microbiota. Therapeutic strategies based on the use of microbial metabolites from the arsenal of competitive interaction have recently appeared using fecal microbiota transplant. They are successfully used to treat recurrent infections caused by *C. difficile* [8, 38, 40].

Inhibitors of bacterial antibiotic resistance mechanisms. In recent years, there appeared many approaches to neutralization of the most effective drug resistance mechanisms in gram-negative bacteria to antimicrobial drugs. Another key mechanism related to overexpression of multiple pumps of active drug efflux has been identified relatively recently [9, 11, 16]. Some of them are already used

in clinical practice, while others are under study, e.g., mechanisms aimed at inhibiting β-lactamases or outflow pumps (efflux pump) [4, 8].

This approach is generally considered to be a promising antibacterial strategy, especially after discovering several natural metabolites and synthetic molecules that inhibit efflux pumps in gram-negative and gram-positive microorganisms [32]. The revival of interest in new drugs, β-lactamase inhibitors, is confirmed by recently emerged new compounds (avibactam, vaborbactam, and relebactam), which are now at different stages of clinical trials [5, 12, 16].

Thus, the above approaches related to resistance inhibition mainly suppress resistance to type II antibiotics making it somewhat risky to rely only on strategies of suppressing evolutionary resistance in the fight against bacterial infections in the future. New strategies for inhibiting the internal resistance of bacteria (type I) are needed. This strategy aimed at destroying the natural mechanisms of antibiotic resistance also includes modulation of functions of small regulatory RNAs (RNA-therapy) [41]. These genetic molecules play a crucial role in controlling biofilm formation, antibiotic resistance, and other bacterial stress reactions, including the formation of dormant forms of bacteria [13, 41]. It is important also that this group of strategies targeted at pathogens does not affect the human microbiota [41].

Among modern innovative genetic antimicrobial strategies, the approach associated with a cluster system with regular intervals of short palindromic repeats (CRISPR) certainly merits attention. Antibacterial drugs grouped at regular intervals based on short palindromic repeats can potentially infect any bacterial pathogen [41–43]. Bacterial CRISPR-Cas9 systems prevent foreign genetic invasions and contain an RNA-gated endonuclease, providing a reliable and multiplexable genome editing tool. This phage-assisted tool can target essential genes or pathogen-specific virulence [42, 43].

Recently, Bikard et al. [43] reported the successful phage-mediated coding of CRISPR-Cas9, which changed the antibiotic resistance of virulent *S. aureus* strains [43]. Since CRISPR technology targets genomes, it will distinguish between pathogens and commensals, which, in its turn, will reduce possible side effects on the microbiota [42, 43].

Nanotechnology as a modern strategy to combat multidrug-resistant (MDR) bacteria. Emerging over the past decades, nanotechnology and its medical applications represent an innovative modern platform for solving the problem of treating infections caused by MDR bacteria. The prefix "nano" refers to any product with properties or phenomena associated with its size in the nanoscale range (1–100 nm) [44, 45]. Nanoparticles and other nanomaterials, the main tools of the nanotechnology industry, have special characteristics that optimize the studied biological, physical, and chemical properties for solving various problems.

Functional and composite nanomaterials based on innovative technologies, the market for which has grown exponentially over the past decades, have unique properties compared to their bulk chemical analogs. For example, a large surface area to volume ratio increases the number of functional sites and can enhance the effect of nanosized particles on a microorganism. In medicine, a high versatility of properties of nanomaterials can improve their antimicrobial action and therapeutic effects and reduce side effects [44, 46–48].

The breadth and versatility of therapeutic applications are some of the most attractive properties of modern medical nanotechnology. It is no coincidence that nanomedicine has always been considered as the science of the future, being one of the actively developing scientific and medical areas. Currently, nanoparticles are successfully used to treat and diagnose various diseases (infectious, oncological, and cardiovascular diseases; thrombosis, osteoporosis, Alzheimer's disease, etc.) [45–47].

Hundreds of billions of dollars have been invested in the global medical nanotechnology market recently. Researchers are attracted by the vast potential and wide possibilities of using nanoparticles as therapeutic agents of a new generation and their use as theranostic agents and modern drug delivery systems [44, 46–48].

Modern applications of nanotechnology in antimicrobial strategies are exciting and promising. The main approaches used are focused on the following areas: i) prevention of bacterial adhesion to prevent biofilm formation; ii) destruction of the formed biofilm and eradication of bacteria without the development of resistance; and iii) the therapeutic effect of nanoparticles on intracellular bacterial pathogens [44, 46]. Let us dwell on each of them in more detail.

Nanomaterials as inhibitors of bacterial adhesion and biofilms formation. Over the past decade, resistance to almost all classical antibiotics and the lack of new antimicrobial molecules have made researchers study the possibility of using nanomaterials for treatment and prevention of microbial infections [45].

The most typical example of this direction is nanostructure transformations of surfaces that prevent biological growth by changing their chemical and/or physical properties. As a result, the new properties of surfaces become highly unfavorable for the attachment of bacteria and subsequent formation of biofilms [45, 47]. This effect is primarily achieved by inhibiting bacterial adhesion upon contact of a bacterial cell with a modified surface [44, 47]. In general, inorganic-based nanomaterials demonstrate significant advantages over their organic counterparts, exhibiting good biocompatibility and higher thermal, chemical, and mechanical stability under physiological conditions [48] (Fig. 2).

Such surface constructions have found their biomedical application in bone tissue regeneration using an implant made using nanomaterials [44, 46, 47]. In this case, in addition to antiadhesive properties, nanoparticles, which have antimicrobial properties by their nature, are successfully used to prevent or combat implant-associated infections. This dangerous complication is a complex infectious process caused mainly by the biofilm-forming pathogenic *Staphylococcus* spp. To a large extent, it is mediated by the initial stage, i.e. the adhesion of bacteria to the implant surface [47, 49].

Bacterial adhesion is conventionally divided into two phases. The initial phase is reversible and is characterized by a nonspecific interaction between the bacterial wall and the implant surface. During the second phase, specific and nonspecific interactions mediated by proteins occur, which leads to irreversible adhesion, subsequent colonization, and biofilm formation [44, 45].

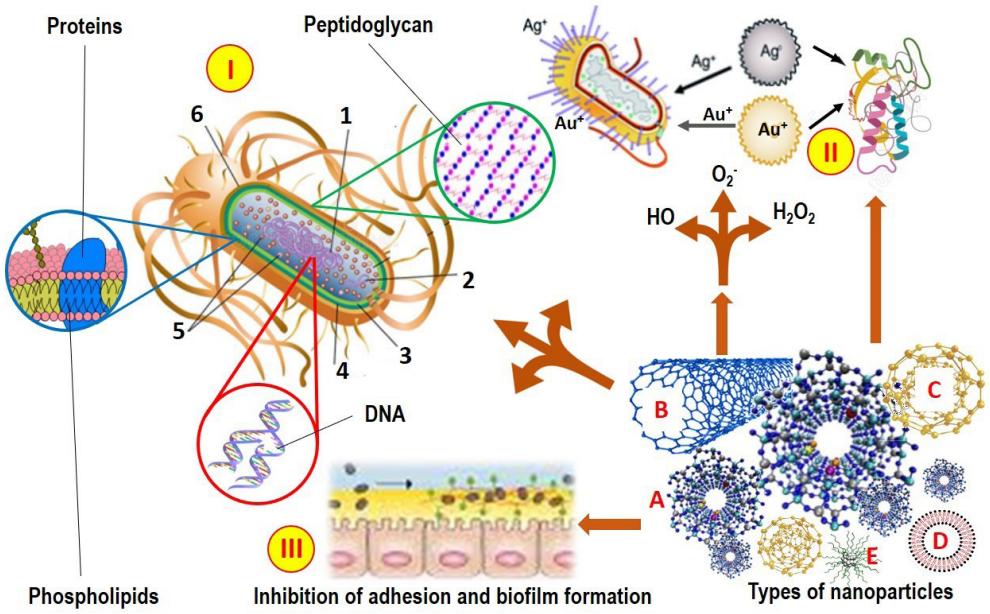

Рис. 2. Основные механизмы и антибактериальные мишени наночастиц
Обозначения: I – паттерны бактериальной клетки: 1 – ядро (ДНК); 2 – цитоплазма;
3 – цитоплазматическая мембрана (интегральные белки и фосфолипиды);
4 – клеточная стенка (пептидогликан); 5 – рибосомы (белковый синтез); 6 – наружная мембрана.
II – механизм токсического действия наночастиц золота и серебра;
III – типы наночастиц: A – наночастицы серебра; B – углеродные нанотрубки;
C – наночастицы золота; D – липосомы; E – полимерная мицелла.

Fig. 2. Basic mechanisms and antibacterial targets of nanoparticles.
I – patterns of a bacterial cell: 1 – nucleus (DNA); 2 – cytoplasm; 3 – cytoplasmic membrane (integral proteins and phospholipids); 4 – cell wall (peptidoglycan); 5 – ribosomes (protein synthesis); 6 – outer membrane.
II – mechanism of toxic action of gold and silver nanoparticles;
III – types of nanoparticles: A – silver nanoparticles; B – carbon nanotubes; C – gold nanoparticles;
D – liposomes; E – polymer micelle.

A biofilm is a complex three-dimensional multicomponent structure formed by many planktonic or aggregated bacteria of separate or mixed species through secretion of extracellular polymeric substances on biotic or abiotic surfaces [45]. Regardless of the type of bacteria and environment, all biofilms share some common properties, including viscoelasticity and the presence of a heterogeneous microenvironment that provides growth, protection, and conditions for the survival of microorganisms. In the process of formation and maturation, biofilm also serves as a mediator of cellular signals and a medium for metabolic activity [44, 45].

A comprehensive analysis of a multicomponent nature of the biofilm has revealed several promising targets for combating microbial infections and infectious diseases [44, 46, 47]. Approaches to inhibiting biofilm formation and destruction can be divided into four classes: i) targeting bacterial adhesion and polymer matrix components; ii) targeting biofilm metabolism; iii) promoting the dispersion of biofilms; and iv) targeting dormant (dormant) cell forms of bacteria [46, 48].

Destruction of the microbial biofilm is a complex and urgent task since impermeability of the biofilm to antimicrobial agents and effector cells of the immune system significantly reduces the effectiveness of treatment. Thus, inhibition of the initial stage of bacterial adhesion to the implant surface is one of the critical strategies for preventing associated bacterial infections [45, 49].

Previously, numerous attempts were made to inhibit biofilm formation and destruction using active molecules targeting bacterial adhesion (e.g., mannoside derivatives) [11], structural protein inhibitors (e.g., 2-pyridones fused to the ring) [14], QS inhibitor peptides (e.g., with the help of autoinductive peptides AIP or RIP) [21], finally, matrix-degrading enzymes (e.g., glucanohydrolase) [9], creating vaccines and using AMPs [32].

However, the most significant antibiofilm effect and high potential after preclinical and clinical trials on models of gram-positive and gram-negative bacteria were shown by the use of metal nanoparticles consisting of gold (Au), silver (Ag), copper (Cu), cerium (Ce), as well as graphene nanosheets and quantum dots (with or without other antibacterial molecules) [45, 47]. Besides, modification of the surface of surgical implants (e.g., silver coating) significantly reduced the likelihood of biofilm formation and increased the life of implants and other biomaterials [48].

Modern nanotechnology offers several anti-adhesive mechanisms for solving this problem. They are primarily associated with surface design changes that inhibit the initial adhesion phase. In addition, surface impregnation with antibiotics, immobilization with bactericidal agents, or coating with antimicrobial components (such as Cu, Ag, titanium dioxide (TiO2), etc.) destroys the approaching bacteria [46, 47].

However, nanobiotechnologists go further. It is not enough to inhibit the adhesion of pathogenic bacteria to prevent infections; it is also necessary to create conditions for improving the adhesion of eukaryotic cells for adequate osseointegration with bone implants, which is essentiall for ensuring their long-term functioning [44]. These conditions are

achieved by chemical modifications providing the zwitterionic (neutral) character of the surface of biomaterials and/or transformation of the coating nanostructure [44, 45].

For example, Vallet-Regн et al. [45] reported the creation of nanostructured mesoporous materials that successfully demonstrated antibacterial and anti-adhesive properties against *S. aureus* as well as good *in vitro* biocompatibility with preosteoblastic cell culture [46]. Another study described a nanobiomaterial based on mesoporous bioceramics that suppressed bacterial adhesion of *Escherichia coli* under conditions of severe inflammation [48].

Nanoparticles as potential means of treatment of bacterial infections. No less exciting results were obtained using nanoparticles to treat bacterial infections associated with intracellular pathogens, which are often inaccessible for traditional antimicrobial agents [44, 47–49].

Thanks to their unique characteristics, nanoparticles act against bacteria through the mechanisms that differ from the standard effects of antibiotic therapy, making nanotechnology extremely effective and promising antimicrobial strategies to which microorganisms usually fail to develop resistance. The spectrum of antibacterial action of nanoparticles is associated with a direct effect on pathogenic bacteria. The implementation of these antibacterial mechanisms is mediated by a spectrum of damaging molecular mechanisms leading to disruption of gene expression and destruction of the bacterial wall [9, 46, 50].

To date, several types of nanoparticles have shown their effectiveness in killing bacteria better than classical antibiotics. In general, the existing spectrum of antimicrobial nanosized particles is quite wide. It can be divided into: i) metal nanoparticles (e.g., nanoparticles of Au, Ag, zinc (Zn) and Cu); ii) polymer nanoparticles (e.g., chitosan nanoparticles); iii) carbon-based nanoparticles (e.g., carbon nanosheets, nanotubes); iv) lipid nanoparticles (e.g. liposomes); v) non-metallic inorganic nanoparticles (e.g., silica nanoparticles); and vi) protein nanoparticles (e.g., albumin nanoparticles) [46, 47].

In particular, metal nanoparticles represent a new potential means of fighting bacteria with fundamentally different action mechanisms. All these nanoforms have some similar advantages, such as small size (less than 10 nm), inert nature, biocompatibility, and biosafety, all making them the means of choice for antibacterial therapy. Certain nanoforms are a vehicle for the delivery of natural antibacterial compounds (e.g., antibiotics), providing another avenue for developing a wide range of powerful antibacterial agents. [48–50]. Some of these nanosized particles have been enhanced with metal ions that are active against prokaryotes to enhance their antibacterial effects.

Among metal nanoparticles, the most impressive results were obtained using silver that is well known for its antibacterial properties. In addition to silver, other metals (e.g., gold) or oxides of zinc, copper, iron, and titanium dioxide, the antimicrobial properties of which are currently being intensively studied, are used in the composition of nanoparticles to treat bacterial infections [44, 49]. As an example, we focused on silver nanoparticles (AgNPs) and gold (AuNPs) and their molecular antibacterial mechanisms of action (Fig. 2-II).

Traditionally, the priority allocation of AgNPs and AuNPs from the group of metal analogs is associated with the potential toxic effects of nanoparticles of Zn, Cu, Ti, Ce, and their oxides on humans and the environment [44, 45], which outweighs their advantages. Therefore, the number of studies of antibacterial properties of nanoparticles containing Au or Ag is extensive and exceeds that of similar studies with other metals [44, 46, 47]. Moreover, modern tools of nanotechnology, chemistry, and biotechnology make it possible to synthesize AuNPs and AgNPs by simple, cheaper, and environmentally friendly methods to study their synergistic action [47].

Extremely small sizes of AuNPs and AgNPs play an important role in providing antimicrobial effects and fighting intracellular bacteria [45]. As a rule, the highest activity is exhibited by 5–13.5-nm silver and 8.4-nm gold nanoforms [44, 45, 47]. Since these nanoparticles act only in contact with bacterial cell walls, electrostatic attraction, Van der Waals (intermolecular) forces, ligand-receptor, and hydrophobic interactions are of great importance [43, 45, 47].

Antimicrobial mechanisms of these nanoparticles are targeted immediately at bacterial cells (by interacting with lipids, LPS, or membrane proteins) and at the biofilm, penetration into which depends on many factors, including its maturity and chemical composition on the obe hand and the size of nanoparticles and their surface charge on the other [44, 46, 47]. After penetration, Ag+ and Au+ ions are leached from nanoparticles, migrate, and interact with cellular elements [44, 47]. Metal ions released from nanoparticles gradually penetrate the microbial cell and interact with amino (-NH), mercapto (-SH), and carboxyl (-COOH) functional groups of proteins and nucleic acids. This interaction results in toxic effects that cause dysregulation of bacterial metabolic processes, intracellular homeostasis, and, ultimately, the death of bacteria [9, 49]. Modern technologies make it possible to modify nanoparticles to achieve a preferable mode of action against one or several types of bacteria using various cytotoxic mechanisms [47].

Induction of the production of reactive oxygen species (ROS) of the main types (hydrogen peroxide, superoxide anions, singlet oxygen, etc.) is catalyzed by metal ions sorbed on nanoparticles. This leads to a disruption of redox homeostasis and a severe oxidative stress damaging cellular macromolecules of bacteria (membrane lipids and proteins, nucleic acids) [9, 47, 50]. The most significant damage to the bacterial cell is caused by singlet oxygen (O_2), which is most active with respect to organic compounds [43]. Oxidative stress is a key factor in changing permeability and damaging of the bacterial wall [44, 47].

AgNPs nanoforms are the most commonly used metal for impregnation of nanomaterials [9, 44, 49]. The revealed multivector antimicrobial effects give grounds for broad prospects for the use of AgNPs in clinical practice and not only as an alternative treatment for infected wounds. For example, Santos et al. [46] appreciated a positive therapeutic effect of these nanoparticles in animals in the postoperative local treatment of caseous lymphadenitis caused by the gram-positive and facultative intracellular pathogen *Corynebacterium pseudotuberculosis* [46].

The widespread use of metal nanoparticles and their oxides in biomedicine is associated with their large and varied potential as therapeutic, diagnostic, cosmetic products and, of course, a powerful antibacterial platform. AuNPs are considered to be relatively safe nanoforms because gold is inert and non-toxic. However, the safety issues of prolonged effects of long-term presence and accumulation of other metal nanoparticles in the body remain unresolved. For example, it is well known that AgNPs can accumulate in various organs of the human body, especially in the brain (overcoming the blood-brain barrier), as well as in the lungs, spleen, kidneys, liver, and brain of rats [43, 47]. Besides, toxic effects of presumably inert nanomaterials based on zinc and titanium dioxide on eukaryotic cells have been observed [45–47].

When assessing future prospects of nanotechnology as the most dynamically and actively developing antimicrobial strategy, it should be concluded that these innovative platforms certainly deserve close attention and further research as an alternative means of preventing and treating bacterial infections. Most positive therapeutic effects have been obtained thanks to implementation of fundamentally new mechanisms of the antimicrobial action of nanoparticles and other nanomaterials.

At this stage of studying the possibility of using medical nanosystems in clinical conditions, it is necessary to conduct additional studies of their biosafety and the absence of cytotoxic effects on eukaryotic cells, which were studied only in vitro on cell cultures.

Conclusion

The increasing resistance of pathogenic microorganisms to antimicrobial drugs is undoubtedly a threat to human existence, so the search and development of new antimicrobial strategies is vitally important. Only some of the most exciting directions and promising approaches to the development of new antibacterial agents are mentioned in this review. Some of them have received approval as pharmaceuticals.

The current crises of bacterial resistance to antibiotics and the paradigm of antibiotic therapy are more of a crisis in the development [5, 11] associated with traditional attempts of certain biotechnology companies or institute groups to solve a global problem using some kind of antimicrobial strategy. In this regard, it is appropriate to turn to eastern wisdom and recall that the Chinese character “weiji” (“crisis”) means both “hazard” and “opportunity”.

There is obviously a “hazard” in the problem of the growing antibiotic resistance. It is connected both with the potential for uncontrollable epidemics of bacterial infections and anxiety for the fate of humankind, which will be resolved in the next 10–15 years before the onset of the post-antibiotic era. I would like to believe that the second meaning of the hieroglyph, “opportunity”, would be realized by humankind following the example of the multivector study and fight against the novel coronavirus disease (COVID-19), including the creation of different types of vaccines in an unprecedentedly short time and the development of new drugs.

The conclusion suggests itself: a global problem needs to be addressed by global efforts including new regulatory guidelines and innovative test designs through transnational and international initiatives supported by public funding and private investments. Besides, government support and creation of interdisciplinary groups of microbiologists, biotechnologists, chemists, biologists, and medical doctors, are necessary to study new antibacterial approaches alternative to traditional antibiotics.

Future solutions might be associated with creation of complex platforms combining two or three antibacterial strategies and identification of priority areas, such as RNA-therapy, immunomodulators/suppressors of bacterial virulence, and the use of innovative nanomaterials with high-performance functions, provided their proven biocompatibility and null toxicity.

The struggle against the increasing resistance of microorganisms to antibiotics requires joint action by public and state institutions, in which the development of safe and effective antibacterial technologies should be combined with the adoption of an international program of strict regulation and strict measures of control over justification and rational use of antibiotics and other antibacterial drugs in medicine, cosmetology, agricultural production, and aquaculture. The main program provisions should become an integral part of the global biosafety policy on the global, national and local levels for active detection and monitoring of the spread of MDR bacteria strains.

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