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M. R. Khara https://orcid.org/0000-0002-6028-9876
O. M. Oleshchuk https://orcid.org/0000-0002-1491-1935
S. M. Marchyshyn https://orcid.org/0000-0001-9585-1251
H. O. Bezkorovayna https://orcid.org/0000-0003-2722-7013
O. O. Iskra https://orcid.org/0009-0005-5960-4292

ANTIBIOTIC RESISTANCE: MOLECULAR MECHANISMS OF FORMATION AND MODERN MANAGEMENT STRATEGIES

Ivan Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine, Ternopil, Ukraine

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M. R. Khara, O. M. Oleshchuk, S. M. Marchyshyn, H. O. Bezkorovayna, O. O. Iskra

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Background. Antibiotic resistance (ABR) is the greatest threat to human survival, leading to a loss of control over resistant bacterial strains. The aim of this work was to demonstrate the pathogenetic basis of ABR and potential solutions to this problem.

Methods. To achieve this goal, an analysis of current scientific publications regarding the mechanisms of ABR and innovative approaches to overcoming it was conducted.

Results. The main mechanisms of ABR include reduced membrane permeability, efflux pumps, modification of antibiotic targets, enzymatic inactivation, horizontal gene transfer, and the involvement of the host's immune system.

Conclusion. Controlling ABR requires the rational use of antibiotics, optimization of traditional antibiotic therapy, modulation of the immune response, microbiome modulation, and phage therapy.

Key words: antibiotic resistance, infection, immune protection, phagocytes, rational antibiotic therapy.

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М. Р. Хара, О. М. Олещук, С. М. Марчишин, Г. О. Безкоровайна, О. О. Іскра

АНТИБІОТИКОРЕЗИСТЕНТНІСТЬ: МОЛЕКУЛЯРНІ МЕХАНІЗМИ ФОРМУВАННЯ ТА СУЧАСНІ СТРАТЕГІЇ МЕНЕДЖМЕНТУ

Тернопільський національний медичний університет імені І. Я. Горбачевського Міністерства охорони здоров'я України, Тернопіль, Україна

Актуальність. Антибіотикорезистентність (АБР) – найбільша загроза виживанню людини, що веде до втрати контролю над резистентними штамами бактерій.

Метою роботи було продемонструвати патогенетичне підгрунтя АБР та можливості вирішення цієї проблеми. Для реалізації мети було проведено аналіз сучасних наукових публікацій щодо механізмів АБР та інноваційних підходів до її подолання.

Результати. Основні механізми АБР передбачають знижену проникність цитолеми, ефлюксні помпи, модифікацію мішеней для антибіотиків (АБ), ферментативну інактивацію, горизонтальний перенос генів, участь імунної системи макроорганізму.

Висновки. Контроль АБР – це раціональне використання АБ, оптимізація традиційної антибіотикотерапії, модуляція імунної відповіді, модуляція мікробіому та фаготерапія.

Ключові слова: антибіотикорезистентність, інфекція, імунний захист, фагоцити, раціональна антибіотикотерапія.

Introduction

Antibiotic resistance is a global public health issue, attracting increasing attention due to its real threat to the human population. Although resistance to antibiotics (ABs) is an inevitable consequence of bacterial evolution and natural selection, the misuse and overuse of ABs in modern medicine and dentistry significantly contribute to the worsening crisis. ABR reduces the ability of medicine to control socially dangerous infections (tuberculosis, syphilis, gonorrhea), and worsens treatment outcomes in patients undergoing chemotherapy, cesarean sections, hip prosthetics, organ transplants, and other complex medical interventions. The problem is exacerbated by uncontrolled antibiotic use in agriculture and the food industry. According to WHO, ABR is a global issue driven by lack of access to clean water, inadequate hygiene and prevention, lack of quality and affordable vaccines, and absence or disregard of legal regulations [1]. For Ukraine, this problem has become particularly acute since July 2022 due to a large number of patients with complex war injuries requiring antibiotic therapy.

Aim of the Study. The aim of the study was to present currently known molecular mechanisms of bacterial adaptation to antibiotics, to elucidate the role of the host organism in the pathogenesis of antibiotic resistance development, and to analyze scientific data on potential strategies for effective targeting of pathogens and enhancing the efficacy of antibiotic therapy using the existing arsenal of drugs.

Materials and Methods
Research results and their discussion

To achieve this aim, a review and integrative analysis of scientific publications available in bibliometric databases was conducted. The selected sources reflect the underlying causes and mechanisms of antibiotic resistance, as well as approaches to addressing this global challenge.

Epidemiology of ABR and Issues Across Medical Fields. The discovery of antibiotics marked a crucial milestone in medicine, saving and continuing to save millions of lives annually. However, their effectiveness is threatened by the ability of bacteria to adapt to hostile environments and diminish or neutralize treatment. ABR arises from numerous mechanisms that enable certain bacteria to survive after the application of specific antibiotics, and the resulting resistance can be passed on to other bacteria as they reproduce [2; 3].

According to the European Centre for Disease Prevention and Control (ECDC), more than 35,000 people die each year in Europe due to ABR [2]. The overall epidemiological situation in the EU remains concerning. Although the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is gradually decreasing, the prevalence of carbapenem-resistant strains of *Klebsiella pneumoniae* has increased by more than 50 % since 2019 [4].

According to WHO, in 2019, ABR caused more than 1.27 million deaths globally [1], and by 2050, this number may exceed 39 million (Lancet prognosis). The economic consequences of such trends include an annual loss of $3.4 trillion and more than 24 million people falling into extreme poverty [5; 6].

The consequences of ABR are already evident in many areas of medicine. Excessive or unjustified use of antibiotics for viral infections contributes to the growth of ABR. This is especially relevant in pediatrics, where about 30 % of antibiotics are prescribed without proper indications or while ignoring local sensitivity data [7].

Chronic respiratory diseases (such as cystic fibrosis and COPD) require prolonged antibacterial treatment, which promotes the development of complex forms of ABR. Traditional susceptibility testing does not always reflect the actual conditions in the lungs, so comprehensive resistome analysis is key to developing personalized therapeutic strategies [8]. Obstetrics and surgery are also affected by the consequences of ABR, as antibiotics are frequently used not only for treatment but also for the prevention of infections [9]. In dermatoveneorology, antibiotics are widely applied to treat socially significant sexually transmitted infections (syphilis, gonorrhea) [10], as well as severe forms of acne, rosacea, and other skin infections [11]. Tuberculosis occupies a distinct place in this issue, being a historical example of both natural and acquired ABR. Treatment of drug-sensitive forms requires the administration of multiple medications over a period of 6–9 months, which is often interrupted by patients, thus promoting the emergence of drug-resistant forms [12; 13]. Drug-resistant tuberculosis (resistant to at least one of the first-line drugs) is transmitted just as easily as drug-sensitive forms, but its diagnosis and treatment are significantly more challenging. The rapid rise in the number of multidrug-resistant forms threatens the global capacity to control this disease [14; 15].

Microorganisms of Concern in Antibiotic Resistance. A significant step in addressing ABR was the establishment by the World Health Organization (WHO) in 2017 of a priority list of the most dangerous antibiotic-resistant microorganisms. This list is divided into three priority tiers: critical, high, and medium. The *critical priority group* includes bacteria posing the highest threat, such as *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant), and members of the *Enterobacteriaceae* family, particularly those producing extended-spectrum β-lactamases (ESBL) or exhibiting carbapenem resistance. These microorganisms are the leading cause of healthcare-associated infections, especially in patients with catheters or on mechanical ventilation. *The high-priority group* includes pathogens that are already widespread in the population and are progressively developing resistance to standard treatment regimens: *Enterococcus faecium* (vancomycin-resistant), *Staphylococcus aureus* (including MRSA and VRSA), *Helicobacter pylori*, *Campylobacter, Salmonella*, and *Neisseria gonorrhoeae*. *The medium-priority group* comprises pathogens that are already showing alarming resistance trends, including *Streptococcus pneumoniae, Haemophilus influenzae*, and *Shigella spp.* [16–18].

ESKAPE Pathogens. Particular attention is currently drawn to a group of pathogens known by the acronym ESKAPE – *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp* [17; 19]. This group of bacteria was named for their ability to 'escape' the effects of available antimicrobial agents, making them some of the most dangerous pathogens in clinical practice [18; 19]. Their survival is due to high genetic plasticity, the ability to horizontally transfer resistance genes, biofilm formation, and resilience in hostile environments, particularly in hospital settings. They are the main representatives of nosocomial infections [20]. The greatest danger is associated with their resistance to last-line ABs, particularly carbapenems. In 2020, every fifth case of urinary tract infections caused by *Escherichia coli* was resistant to standard AB. *Klebsiella pneumoniae* also demonstrates growing antimicrobial resistance. This forces doctors to resort to reserve ABs, which accelerates the formation of resistant strains [18]. In addition, the spread of new invasive pathogens, such as *Candida auris* – a fungus resistant to several classes of antifungal drugs, capable of causing outbreaks of nosocomial infections – is increasing [21; 22].

Interestingly, ESKAPE bacteria, which are responsible for the development of bacteremia, ventilator-associated pneumonia, endocarditis, and infections of the urinary tract, skin, and soft tissues, do not always behave as pathogens. They can colonize the skin or mucous membranes without causing disease. Problems arise when these microbes enter sterile areas of the body, such as the blood or cavities, where they quickly cause severe, often treatment-resistant infections. The unique ability of ESKAPE pathogens to evade AB action and colonize a wide range of environments makes them the main drivers of the modern ABR crisis [23].

Types and Mechanisms of ABR. ABR exists in natural and adaptive forms. Natural ABR can be intrinsic or induced.

Intrinsic ABR is always universal within a bacterial species, doesn't depend on prior exposure to ABs, and isn't linked to horizontal gene transfer. Induced ABR is initiated by genes consistently present in bacteria, but their expression to ABR levels is triggered by ABs [24; 25]. The most common mechanisms of intrinsic ABR are reduced permeability of the bacterial outer membrane (due to lipopolysaccharides in Gram-negative bacteria) and the natural activity of AB efflux pumps, which are also a common mechanism of induced AMR [25; 26]. Acquired ABR develops through the acquisition of genetic material via all major pathways – transformation, transposition, and conjugation – collectively known as horizontal gene transfer (HGT). Additionally, bacteria can undergo mutations in their own chromosomal DNA. In this case, ABR can be temporary or permanent [27]. Adaptive AMR occurs when the amount of ABs increases during treatment; it's characterized by rapid resistance development and a swift return to a non-resistant phenotype after the AB is removed from the environment [28]. The mechanisms of ABR in pathogenic bacteria form the basis of multidrug resistance. Depending on the chemical structure of the AB, bacteria employ various counteracting mechanisms, including low outer membrane permeability, drug efflux pumps, AB inactivation, and modification of drug targets [29; 30].

Low Permeability of the Outer Membrane is a survival mechanism that microorganisms developed through evolution to protect themselves from toxic substances, including natural ABs. This mechanism involves a complex located on the outer and inner membranes of bacteria. This complex consists of lipid A, an oligosaccharide core, and O-antigen, which act as recognizers of anything foreign outside the bacterium. Through stabilization by Ca and Mg ions, lipopolysaccharide molecules connect, forming negatively charged phosphate groups that repel hydrophobic AB molecules. Hydrophobic ABs (like rifampicin and fluoroquinolones) can penetrate this lipid bilayer by diffusion. However, for hydrophilic ABs and nutrients, access is only possible through porin channels. These porins are less common in *mycobacteria* and allow for significantly lower uptake rates than in Gram-negative bacteria, which substantially limits drug entry [31].

Efflux pumps are another key mechanism in the development of ABR. These are transport proteins (transporters) involved in eliminating toxic substances from bacteria. These proteins are found in Gram-positive and Gram-negative bacteria, as well as in eukaryotes. There are five main families of efflux transporters: MF, MATE, RND, SMR, and ABC. The ABC family is powered by ATP hydrolysis, while all other families use a proton gradient [32]. For example, the cell wall of *P. aeruginosa* bacteria contains two efflux pumps: MexAB-OprM and MexXY. The MexAB-OprM pump spans both the inner and outer membranes of the bacterium and effectively expels toxins and ABs from the cell, reducing their concentration to an ineffective level. This complex consists of three key components: MexB – an RND family protein embedded in the inner membrane, responsible for recognizing and transporting harmful substances; MexA – a periplasmic protein that connects MexB to the outer channel; OprM – an outer membrane protein that forms the exit channel. The MexXY pump plays an important role in P. aeruginosa's resistance, specifically to aminoglycosides. Its unique feature is that this protein complex does not have its own outer membrane channel. Instead, it utilizes OprM from the MexAB-OprM complex and is activated by the inactivation of the MexZ repressor, which in turn activates the MexXY gene. This leads to the active elimination of ABs by the MexXY/OprM pump, which lowers their concentration inside the bacterium and renders the ABs ineffective [33].

Porin Channels. In bacteria, especially Gram-negative ones with a large outer membrane, many substances, including ABs, enter the cell through special protein channels called porins. Typically, these channels allow hydrophilic molecules to pass through. Bacteria develop ABR by affecting porins in two main ways. First one, decreasing the number of porins or blocking their synthesis: Bacteria can reduce the total number of porins in their outer membrane or block the production of specific types. Members of the *Enterobacteriaceae* family often use this mechanism to acquire ABR, particularly to carbapenems. The fewer "entry gates" there are, the less AB can get into the bacterium. And second one, initiating mutations that alter porin channel structure: This method either reduces or completely prevents the entry of certain ABs while still allowing essential nutrients to pass. For example, such mutations in *E. aerogenes* have caused ABR to imipenem and some cephalosporins. Similarly, in *Neisseria gonorrhoeae*, a mutation in loop 3 of the penB locus led to resistance against beta-lactams and tetracycline [34; 35].

Induction of Chromosomally-Encoded β-Lactamases. When there's a downregulation or absence of outer membrane OprD porins, beta-lactam antibiotics can still get into the bacterial cell. Once inside, they can induce the chromosomally-encoded β-lactamase AmpC. This essentially "turns on" the AmpC gene, leading to the production of a large amount of inducible cephalosporinase, which then breaks down the ABs. Additionally, the blaOXA-50 gene is consistently produced in bacteria, encoding a constitutively expressed oxacillinase (OXA-50). This enzyme also destroys ABs that are susceptible to its action [36]

Horizontal gene transfer is a mechanism through which ABR genes spread among bacteria. It involves the transmission of genetic material to a bacterium that is not its offspring via conjugation, transformation, or transduction. *Conjugation* is a contact-dependent process whereby mobile genetic elements, such as plasmids, integrative and conjugative elements (ICEs), are transferred through a pilus or pore between adjacent bacteria, either within the same genus or across different species. This enables the rapid dissemination of ABR genes. *Transformation* involves the uptake by recipient bacteria of extracellular DNA released from donor bacteria lysed by antibiotics. Once absorbed, this DNA integrates into the recipient bacterium's genome, conferring new traits, including ABR. *Transduction* refers to the use of a temperate bacteriophage as a vector for transferring both chromosomal and extrachromosomal DNA from donor to recipient bacteria, thereby conferring new characteristics [37].

Modification of antibiotic targets. Bacteria possess numerous cellular components that serve as targets for

antibiotics. In the case of ABR, the quantity and/or structure of penicillin-binding proteins in the bacterial cell wall and membrane are altered due to the acquisition of van genes. Changes also occur in the structure of peptidoglycans and the surface charge of the bacterial membrane, which may become more positive. In ribosomes, mutations can lead to methylation of the ribosomal subunit or the protection of ribosomes by specific proteins. Target modification also encompasses metabolic alterations, including the synthesis of nucleic acids through changes in DNA gyrase or topoisomerase IV, and altered metabolism due to mutations in genes encoding enzymes involved in folate synthesis (DHPS, DHFR) [27; 38].

Inactivation of ABs. Bacteria have developed effective strategies to counteract ABs, among which inactivation plays a key role. This process occurs either through hydrolysis of the AB by β-lactamases [38–40], or via the chemical modification of antibiotics by the addition of acetyl, phosphoryl, or adenylyl groups, which renders the compound inactive [27].

Biofilm formation. Due to the protective properties of their matrix and physiological changes in the cells – leading to the development of metabolically dormant cells – biofilms exhibit exceptional resistance to various chemical and physical environmental factors. Initially, planktonic microorganisms reversibly adhere to biological surfaces within seconds of contact. This is followed, within minutes, by irreversible attachment of the microorganisms. The subsequent stage of biofilm formation involves cell growth and division, occurring over the course of several hours. In the following days, exopolysaccharides are produced, and water channels begin to form. These structures hinder the penetration of ABs through the mucosal surfaces of organs and into the deeper layers of the biofilm. The slow diffusion of ABs through the biofilm, combined with the action of bacterial enzymes and chemical substances, results in the inactivation and degradation of the ABs. Over the ensuing months, additional bacteria adhere to the biofilm layers and disperse across its surface. Moreover, bacteria within biofilms utilise horizontal gene transfer mechanisms, which further enhance their resistance to ABs [41].

*The Role of the Host Organism in the Development of ABR.*The host immune system, particularly phagocytic cells, plays a crucial role in the pathogenesis of ABR. Macrophages and neutrophils employ a wide range of antibacterial mechanisms, including reactive oxygen species (ROS), reactive nitrogen species (NO), proteolytic enzymes (e.g., cathepsins, lysozyme), antimicrobial peptides (such as LL-37), and phospholipases. The generation of ROS and RNS is mediated by the enzyme systems Nox2 and NOS2, respectively. The products of these reactions, including peroxynitrite, can damage bacterial cells but at the same time contribute to the development of ABR by inducing a state of metabolic dormancy [42]. In *Staphylococcus aureus*, for instance, peroxynitrite disrupts the function of aconitase, thereby blocking the tricarboxylic acid cycle and driving the bacterium into a therapy-resistant state. Similar effects have been observed in infections caused by *Mycobacterium tuberculosis* and *Salmonella typhimurium*, indicating a potentially universal mechanism. Neutrophil extracellular traps (NETs) and macrophage extracellular traps (mETs) further contribute to bacterial localisation and are essential for pathogen control under conditions where classical phagocytosis is impaired [43]. Of particular concern is the global spread of methicillin-resistant *S. aureus* (CA-MRSA), which is epidemiologically associated with severe skin infections and pneumonia, largely due to its high virulence. A major contributing factor is the strong selective pressure exerted by ABs on *S. aureus*, which activates both horizontal and vertical gene transfer mechanisms, leading to resistance against penicillins and structurally related β-lactam antibiotics. Phenol-soluble modulins of the alpha-type (PSMα), a novel group of S. aureus peptides, recruit, activate, and ultimately lyse human neutrophils, increasing bacterial virulence and reducing the efficacy of antimicrobial therapy [44]. Moreover, *S. aureus* is capable of evading the host immune response partly due to the anti-inflammatory cytokine IL-10, which promotes biofilm formation under conditions of macrophage anti-inflammatory programming (into the M2 subpopulation) [45]. *Mycobacterium tuberculosis* exploits both resident and recruited macrophages for survival, ultimately inducing their death. The released bacterial aggregates can evade phagocytosis and destroy macrophages in a contact-dependent manner by disrupting the plasma membrane, inducing calcium accumulation in the cytosol, and triggering pyroptosis. These effects are dependent on the Mtb ESX-1 secretion system and the presence of the surface lipid phthiocerol dimycocerosate [46].

Overcoming of the ABR Problem. Tackling ABR is a multifaceted challenge due to the complexity and diversity of resistance mechanisms. One promising approach under active investigation is the modulation of the host immune response. For instance, it has been demonstrated that streptazolin – a natural compound derived from *Streptomyces* – enhances the phagocytic activity of macrophages by activating the NF-κB signalling pathway, stimulating ROS production, and inducing cytokine secretion. Bosutinib, a SRC kinase inhibitor, has been shown to further augment phagocytic activity, ROS generation, and macrophage survival, thereby improving their capacity to eliminate resistant strains such as vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*. The combined application of bosutinib and mitoxantrone has demonstrated an additive effect [47]. Antioxidants such as N-acetylcysteine (NAC) and tempol help to reduce oxidative stress and enhance AB efficacy, particularly against intracellular pathogens. Furthermore, immunomodulatory agents – including PPARγ agonists and glucocorticoids – facilitate macrophage adaptation for more effective pathogen clearance [48]. In one study, a nanoparticle named COSBN@CFS@PS was developed to combat recurrent infections caused by intracellular, antibiotic-resistant bacteria. This nanoparticle is pH- and H_2O_2-responsive, allowing for targeted action within macrophages. It is composed of chitosaccharides (COS), cefoxitin (CFS) – a β-lactam antibiotic – and a pinacol ester of phenylboronic acid (a β-lactamase inhibitor). The surface coating of phosphatidylserine (PS) facilitates the nanoparticle's uptake by macrophages. Once inside the macrophage, under acidic conditions and in the

presence of H_2O_2, the nanoparticle releases boronic acid, which inhibits β-lactamase and prevents degradation of the AB. This targeted delivery system significantly enhances the antibacterial efficacy of cefoxitin in the treatment of intracellular infections [49].

Drug repositioning is another promising approach in the fight against ABR. This strategy involves identifying new therapeutic applications for existing, already-approved drugs originally developed for other diseases. One particularly interesting direction is the investigation of antibiotics, traditionally used to treat bacterial infections, for their potential antiviral properties. Studies have indicated that certain classes of ABs, including macrolides, glycopeptides, tetracyclines, fluoroquinolones, and aminoglycosides, may exhibit antiviral activity against a range of RNA and DNA viruses, including SARS-CoV-2. Although the mechanisms underlying this antiviral activity are not yet fully understood, they are thought to involve both direct effects on the viral particles and indirect effects such as the immunomodulatory and anti-inflammatory properties of these ABs, which may influence the host's response to viral infection. Despite the promising nature of these findings, the use of antibiotics to treat viral infections remains controversial and is not currently accepted as standard medical practice. Robust clinical trials are required to confirm the efficacy and safety of antibiotics in this novel role. To date, no antibiotic has been officially approved for use as an antiviral agent [50].

Optimisation of Existing Antibiotics. In addition to the development of new drugs, considerable efforts are being directed towards the optimisation of treatment regimens using existing, or "old", antibiotics. In the United States, the National Institute of Allergy and Infectious Diseases (NIAID) is actively conducting research focused on refining the use of established ABs. This includes exploring novel routes of administration, determining the most effective and shortest possible duration of therapy, and evaluating combination treatment strategies – specifically, the concurrent use of two or more antibiotics [51; 52].

Microbiome-Based Approaches. Researchers are exploring non-traditional strategies for treating antibiotic-resistant infections, including the development of live microbiome-based therapeutics. Scientists from the NIAID have collaborated with researchers in Thailand on a project demonstrating that *Bacillus* – a "beneficial" bacterium commonly found in probiotic digestive supplements – can aid in eliminating *Staphylococcus aureus*. NIAID is also investigating the use of faecal microbiota transplantation as a treatment for recurrent *Clostridium difficile*-associated disease (CDAD), a potentially life-threatening form of diarrhoea. This procedure involves introducing stool from a healthy donor – screened for infectious agents and antibiotic-resistant organisms – into the recipient's colon to restore a healthy and diverse gut microbiome [53].

Phage Therapy. Bacteriophages (phages) are viruses that selectively infect and destroy bacteria. Phage therapy has been employed to treat patients suffering from severe, multidrug-resistant infections under compassionate use protocols, with encouraging outcomes. However, significant knowledge gaps currently hinder the development, standardisation, and regulatory approval of phage-based treatments [54]. While the complete elimination of antimicrobial resistance is unachievable – given that it is a natural evolutionary process – modifying our approaches to antibiotic use can have a substantial impact in slowing the spread of this global threat [55].

Adjuvant Coupling: Synergistic Therapy. The usage of adjuvants enables the restoration of bacterial sensitivity to existing antibiotics by blocking resistance mechanisms (e.g., efflux pumps) or facilitating drug penetration into the cell. Combining antibiotics with mucolytics (e.g., N-acetylcysteine) and enzybiotics (peptidoglycan-degrading enzymes) is effective for the disruption of bacterial biofilms. This allows the antimicrobial agent to penetrate the deep layers of the infectious focus [56].

Discovery of New Classes: Lantibiotics. It is a class of ribosomally synthesized antimicrobial peptides (bacteriocins) containing unconventional amino acids (lanthionines). They possess a unique, typically dual mechanism of action: binding to lipid II, which disrupts cell wall synthesis, and pore formation in the membrane. This renders the development of resistance extremely difficult. Representatives of this class, such as Nisin and the novel lichenicidins, are considered potent alternatives for combating methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) [57].

Rational Use of Antibiotics in Medical Practice. To combat the growing threat of antibiotic resistance, the WHO has developed key strategies aimed at promoting the rational use of antibiotics [58]. In our view, the most essential and effective measures for healthcare systems include the following:

1. *Optimal dosing and treatment duration.* Inadequate antibiotic dosing or premature discontinuation of therapy may result in the survival and subsequent proliferation of resistant bacterial strains. Conversely, excessive use increases the risk of adverse effects for the patient and promotes the development of ABR [59].

2. *Use of antibiotics only when clinically indicated.* Antibiotics should be prescribed exclusively in cases where a bacterial infection has been confirmed or is highly suspected. Their use in viral illnesses – such as the common cold or influenza – is entirely inappropriate and counterproductive.

3. *Expansion of infection prevention systems.* The most effective way to reduce the need for antibiotics is to prevent infections in the first place. Vaccination (e.g., against influenza or pneumococcal disease) and proper hand hygiene play critical roles in this regard.

4. *Monitoring of ABR.* Systematic collection and analysis of data on microbial susceptibility allow for the adjustment of antibiotic prescribing guidelines at both local and national levels, enabling timely responses to evolving resistance patterns.

Regulatory Framework for Rational Antibiotic Therapy in Ukraine. Ukraine, recognising the global challenges posed by antimicrobial resistance, has taken active steps to promote the rational use of ABs. One of the most significant regulatory measures is the Order of the Ministry of Health of Ukraine No. 1513, dated 23 August 2023, entitled "*On the Approval of the Standard of Medical Care: Rational Use of Antibacterial and Antifungal*

Agents for Therapeutic and Prophylactic Purposes." This order serves as a foundational document, marking a substantial shift in the approach to prescribing and using antimicrobial agents within the Ukrainian healthcare system. Its adoption represents a crucial step in aligning Ukrainian legislation with international and European standards in the fight against ABR. The document clearly regulates the prescription of ABcs, emphasising the importance of using clinical protocols and microbiological test results, and restricts the uncontrolled use of broad-spectrum antibiotics. Furthermore, it introduces a system for ABR monitoring, enabling the collection, analysis, and exchange of data on microbial susceptibility – an essential element for formulating up-to-date recommendations and detecting emerging threats. In addition, the order establishes requirements for detailed accounting of antibiotic consumption and mandates regular reporting. The implementation of this regulation aims not only to reduce inappropriate antibiotic use and slow the progression of ABR but also to improve the quality of infectious disease treatment and alleviate the financial burden on the healthcare system.

Another critically important regulatory act addressing the rational use of antibiotics in wartime conditions in Ukraine is the Ministry of Health's Order No. 1004, dated 1 June 2023, entitled "*On Approval of the Standard of Medical Care 'Prevention of Infectious Complications of Combat Injuries with Antibacterial Drugs at the Pre-Hospital Stage*.'" This order holds exceptional relevance in the context of the ongoing full-scale military aggression against Ukraine. It governs the use of antibiotics under the extreme conditions of armed conflict, where timely and appropriate administration can be vital for saving the lives and preserving the health of the injured. The document clearly defines the circumstances under which prophylactic antibiotic use is justified and necessary already at the pre-hospital stage for patients with various types of wounds. It specifies the recommended antibiotics for prophylactic use, based on their spectrum of activity against the most likely pathogens associated with combat-related infections, and outlines the optimal routes of administration under field conditions. The primary goal of such prophylactic application is to minimise the risk of purulent-septic complications, which are common in combat trauma due to the high level of wound contamination. Early antibiotic intervention can prevent infection from spreading or becoming systemic.This order is of critical importance for safeguarding the lives and health of wounded military personnel and civilians, as timely and evidence-based antibiotic use in the pre-hospital phase can significantly improve clinical outcomes, reduce the need for complex surgical interventions, and shorten rehabilitation periods.

According to the Order of the Ministry of Health of Ukraine No. 1614 dated August 3, 2021, "On the Organization of Infection Prevention and Control in Healthcare and Social Assistance/Social Protection Institutions," a multidisciplinary team must be established in every hospital to implement the antimicrobial resistance containment strategy. This team must necessarily include a clinical pharmacist, who is responsible for the following processes:

- *Pre-authorization:* Approval of the prescription of "Reserve" group antibiotics;
- *Prospective Audit and Feedback:* Real-time review of prescriptions regarding their appropriateness, dosage, and duration;
- *Consultative Support:* Providing recommendations to physicians on drug selection based on the facility's local antimicrobial resistance profile;
- *Educational Function:* Training staff on the principles of rational antibiotic therapy.

These and other regulatory acts issued by the Ministry of Health of Ukraine form a robust foundation for a systematic approach to antibiotic therapy management. They integrate the principles of rational antibiotic use into routine clinical practice and ensure effective control over the circulation of these vital medications. The implementation of antimicrobial stewardship programs involves the coordinated efforts of physicians and clinical pharmacists. This ensures continuous oversight of the appropriateness of prescriptions, the selection of the optimal drug, and the monitoring of treatment efficacy at all stages of healthcare system.This is especially critical in the context of wartime, where the resilience of the healthcare system and the ability to effectively manage infectious complications are of paramount importance.

Conclusions

Antibiotic resistance represents a global public health crisis, driven both by the natural evolution of bacteria and the irrational use of antibiotics. Bacteria have developed sophisticated defence mechanisms, including reduced membrane permeability, active efflux of antibiotics, target site modification, enzymatic inactivation, horizontal gene transfer of resistance traits, and biofilm formation. Simultaneously, the human host, through complex inflammatory and immune responses, may also contribute to the emergence and persistence of antimicrobial resistance. Effective response to this threat requires a comprehensive and multifaceted approach. This includes modulation of the immune response, development of new drugs and pharmaceutical formulations, and the assessment and optimisation of existing antibiotic therapies. Microbiome-based strategies – such as the use of probiotics and faecal microbiota transplantation – alongside phage therapy, represent promising adjunctive approaches. Crucially, the rational use of antibiotics in medical practice remains a cornerstone of antibiotic resistance control. This involves appropriate dosing, treatment duration, and prescription based strictly on clinical indications. Infection prevention measures and state-level regulatory oversight, including the implementation of modern antibiotic resistance monitoring systems, are essential.The adoption of these strategies is vital not only for maintaining the effectiveness of antibacterial therapies but also for safeguarding global public health security in the face of an escalating antimicrobial resistance threat.

BIBLIOGRAPHY

1. World Health Organization. Antimicrobial resistance. Geneva: WHO; 2023. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
2. European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance data for 2024 Antimicrobial resistance (AMR) reporting protocol 2025. http://ecdc.europa.eu/sites/default/files/documents/EARS-Net-reporting-protocol.pdf.
3. Habboush Y, Guzman N. Antibiotic Resistance. [Updated 2023 Jun 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan. https://www.ncbi.nlm.nih.gov/books/NBK513277/.
4. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net). Annual Epidemiological Report 2023. Stockholm: ECDC; 2024. https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-eueea-ears-net-annual-epidemiological-report-2023.
5. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. *Lancet*. 2024.404(10459):1199–1226. DOI: 10.1016/S0140-6736(24)01867-1.
6. Martin JS, Genyn H. One Planet, One Health: Unpacking the Relationship between Antimicrobial Resistance & Climate Change. RM. *Global health*. 2024. https://rabinmartin.com/insights/unpacking-the-relationship-between-antimicrobial-resistance-climate-change/?gad_source=1&gclid=Cj0KCQjw2N2_BhCAARIsAK4pEkUVgMTPoBBIjZ_l62jiikhg0UgPKvJsQ89WaNJCFXBPgWwsLk4klJ0aAoD_EALw_wcB.
7. About Antimicrobial Resistance. Centers for Disease Control and Prevention. 2024. https://www.cdc.gov/antimicrobial-resistance/about/index.html.
8. Pailhoriès H, Herrmann JL, Velo-Suarez L, Lamoureux C, Beauruelle C, et al. Antibiotic resistance in chronic respiratory diseases: from susceptibility testing to the resistome. European Respiratory Review. 2022;31(164):210259. DOI: 10.1183/16000617.0259-2021.
9. Schaefer M, Lui A, Ramesh P, Little C, Megli C. Determinants of antimicrobial resistance in a contemporary obstetrical cohort. *American Journal of Obstetrics and Gynecology*. 2022;226(1,S142). DOI: 10.1016/j.ajog.2021.11.250.
10. Chandra S, Broom A, Ridge D, et al. Treatment 'cultures', sexually transmitted infections and the rise of antimicrobial resistance. *Sociol Health Illn*. 2025;47(1):e13832. DOI: 10.1111/1467-9566.13832.
11. Shah RA, Hsu JI, Patel RR, et al. Antibiotic resistance in dermatology: The scope of the problem and strategies to address it. *Journal of the American Academy of Dermatology*. 2022;86(6):1337–1345. DOI: 10.1016/j.jaad.2021.09.024.
12. Nguyen L. Antibiotic resistance mechanisms in M. tuberculosis: an update. *Archives of Toxicology*. 2016;90(7):1585–1604. DOI: 10.1007/s00204-016-1727-6.
13. Osei Sekyere J, Reta MA, Maningi NE, Fourie PB. Antibiotic resistance of Mycobacterium tuberculosis complex in Africa: A systematic review of current reports of molecular epidemiology, mechanisms and diagnostics. *Journal of Infection*. 2019;79(6):550–571. DOI: 10.1016/j.jinf.2019.10.006.
14. Liebenberg D, Gordhan BG, Kana BD. Drug resistant tuberculosis: Implications for transmission, diagnosis, and disease management. Frontiers in Cellular and Infection *Microbiology*. 2022;12:943545. DOI: 10.3389/fcimb.2022.943545.
15. Floyd K, Glaziou P, Houben RMGJ, Sumner T, White RG, Raviglione M. Global tuberculosis targets and milestones set for 2016-2035: definition and rationale. *International Journal of Tuberculosis and Lung Disease.* 2018;22(7):723–730. DOI: 10.5588/ijtld.17.0835.
16. Chesnokova MM, Bazhora YI, Antonenko KO, Ostapchuk KV. The pecularities of tuberculosis, caused by Beijing strains. *Odesa Medical Journal*. 2022;1–2:55–59. DOI: 10.54229/2226-2008-2022-1-2-10.
17. Antonenko KO, Kresyun VI, Antonenko, PB. Mutations leading to drug-resistant Mycobacterium tuberculosis infection in Ukraine. *Central European Journal of Medicine.* 2010;5(1):30–35. https://doi.org/10.2478/s11536-009-0114-6.
18. Alvarez-Ainza ML, Fong-Coronado PA, Ruiz-Bustos E et al. Antibiotic resistance of ESKAPE group-microorganisms in health institutions from Hermosillo and Ciudad Obregon, Sonora, Mexico. *Frontiers in Cellular and Infection Microbiology.* 2024;14. DOI: 10.3389/fcimb.2024.1348093.
19. Benkő R, Gajdács M, Matuz, M, et al. Prevalence and antibiotic resistance of ESKAPE pathogens isolated in the emergency department of a tertiary care teaching hospital in Hungary: A 5-year retrospective survey. *Antibiotics*. 2020;9:1–17. DOI: 10.3390/antibiotics9090624.
20. Boucher HW, Talbot GH, Bradley JS, et al. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clinical Infectious Diseases.* 2009;48(1):1–12. https://doi.org/10.1086/595011.
21. Hu Y, Liu C, Shen, Z, et al. Prevalence, risk factors and molecular epidemiology of carbapenem-resistant Klebsiella pneumoniae in patients from Zhejiang, China, 2008–2018. *Emerging Microbes & Infections.* 2020;9:1771–1779. DOI: 10.1080/22221751.2020.1799721.
22. Lone SA, Ahmad A. Candida auris-the growing menace to global health. Mycoses. 2019;62(8):620–637. DOI: 10.1111/myc.12904.
23. Denissen J, Reyneke B, Waso-Reyneke M, et al. Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. *International Journal of Hygiene and Environmental Health*. 2022l;244:114006. DOI: 10.1016/j.ijheh.2022.114006.
24. Martinez JL. General principles of antibiotic resistance in bacteria. *Drug Discovery Today*. 2014;11:33–39. DOI: 10.1016/j.ddtec.2014.02.001.
25. Cox G, Wright GD. Intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *International Journal of Medical Microbiology*. 2013;303:287–292. DOI: 10.1016/j.ijmm.2013.02.009.
26. Fajardo A, Martinez-Martin N, Mercadillo M, et al. The neglected intrinsic resistome of bacterial pathogens. PLoS One. 2008;3:e1619. DOI: 10.1371/journal.pone.0001619.

27. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*. 2018;4(3):482–501. DOI: 10.3934/microbiol.2018.3.482.
28. Motta SS, Cluzel P, Aldana M. Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps. *PLoS One*. 2015;10(3):e0118464. DOI: 10.1371/journal.pone.0118464.
29. Cag Y, Caskurlu H, Fan Y, et al. Resistance mechanisms. *Annals of Translational Medicine*. 2016;4(17):326. DOI: 10.21037/atm.2016.09.14.
30. Devi NSh, Mythili R, Cherian T, et al. Overview of antimicrobial resistance and mechanisms: The relative status of the past and current. *The Microbe*. 2024;3:100083. https://doi.org/10.1016/j.microb.2024.100083.
31. Manrique PD, Leus IV, López CA, et al. Predicting permeation of compounds across the outer membrane of P. aeruginosa using molecular descriptors. *Communications Chemistry.* 2024 Apr 12;7(1):84. DOI: 10.1038/s42004-024-01161-y.
32. Alenazy R. Drug Efflux Pump Inhibitors: A Promising Approach to Counter Multidrug Resistance in Gram-Negative Pathogens by Targeting AcrB Protein from AcrAB-TolC Multidrug Efflux Pump from *Escherichia coli*. *Biology (Basel).* 20228;11(9):1328. DOI: 10.3390/biology11091328.
33. Avakh A, Grant GD, Cheesman MJ, et al. The Art of War with *Pseudomonas aeruginosa*: Targeting Mex Efflux Pumps Directly to Strategically Enhance Antipseudomonal Drug Efficacy. *Antibiotics (Basel).* 2023;12(8):1304. DOI: 10.3390/antibiotics12081304.
34. Blair JM, Richmond GE, Piddock LJ. Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. *Future Microbiology.* 2014;9:1165–1177. DOI: 10.2217/fmb.14.66.
35. Gill MJ, Simjee S, Al-Hattawi K, et al. Gonococcal resistance to β-lactams and tetracycine involves mutation in loop 3 of the porin encoded at the penB locus. *Antimicrobial Agents and Chemotherapy*. 1998;42:2799–2803. DOI: 10.1128/aac.42.11.2799.
36. Girlich D, Naas T, Nordmann P. Biochemical Characterization of the Naturally Occurring Oxacillinase OXA-50 of Pseudomonas aeruginosa. *Antimicrobial agents and Chemotherapy.* 2004;48:2043–2048. DOI: 10.1128/AAC.48.6.2043-2048.2004.
37. Tao S, Chen H, Li N, et al. The Spread of Antibiotic Resistance Genes In Vivo Model. *Canadian Journal of Infectious Diseases and Medical Microbiology.* 2022;2022:3348695. DOI: 10.1155/2022/3348695.
38. Thacharodi A, Lamont IL. Aminoglycoside-Modifying Enzymes Are Sufficient to Make *Pseudomonas aeruginosa* Clinically Resistant to Key Antibiotics. *Antibiotics (Basel).* 2022;11(7):884. DOI: 10.3390/antibiotics11070884.
39. Vivekanandan KE, Kumar PV, Jaysree RC, Rajeshwari T. Exploring molecular mechanisms of drug resistance in bacteria and progressions in CRISPR/Cas9-based genome expurgation solutions. *Global Medical Genetics.* 2025 Feb 16;12(2):100042. DOI: 10.1016/j.gmg.2025.100042.
40. Blair JM, Webber MA, Baylay AJ, et al. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology.* 2015;13(1):42–51. DOI: 10.1038/nrmicro3380.
41. Liu HY, Prentice EL, Webber MA. Mechanisms of antimicrobial resistance in biofilms. *NPJ Antimicrobials and Resistance.* 2024;2(1):27. DOI: 10.1038/s44259-024-00046-3.
42. Sun K, Yajjala VK, Bauer C, et al. Nox2-derived oxidative stress results in inefficacy of antibiotics against post-influenza S. aureus pneumonia. *Journal of Experimental Medicine.* 2016;213(9):1851–1864. DOI: 10.1084/jem.20150514.
43. Flannagan RS, Heit B, Heinrichs DE. Antimicrobial Mechanisms of Macrophages and the Immune Evasion Strategies of Staphylococcus aureus. *Pathogens*. 2015;4(4):826–868. DOI: 10.3390/pathogens4040826.
44. Chambers HF, DeLeo FR. Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era. *Nature Reviews Microbiology.* 2009;7(9):629–641. DOI: 10.1038/nrmicro2200.
45. Bertrand BP, Shinde D, Thomas VC, Whiteley M, Ibberson CB, Kielian T. Metabolic diversity of human macrophages: potential influence on *Staphylococcus aureus* intracellular survival. *Infection and Immunity*. 2024 Feb 13;92(2):e0047423. DOI: 10.1128/iai.00474-23.
46. Toniolo Ch, Dhar N, McKinney JD. Uptake-independent killing of macrophages by extracellular *Mycobacterium tuberculosis* aggregates. *EMBO Journal*. 2023;42(9):e113490. DOI: 10.15252/embj.2023113490.
47. Dai X, Li Y, Zhang Y, et al. pH/H_2O_2 dual-responsive macrophage-targeted chitosaccharides nanoparticles to combat intracellular bacterial infection. *Colloids and Surfaces B: Biointerfaces*. 2025;248:114465. DOI: 10.1016/j.colsurfb.2024.114465.
48. da Silva RAG, Stocks CJ, Hu G, et al. Bosutinib Stimulates Macrophage Survival, Phagocytosis, and Intracellular Killing of Bacteria. *ACS Infectious Diseases.* 2024;10(5):1725–1738. DOI: 10.1021/acsinfecdis.4c00086.
49. Beam JE, Rowe SE, Conlon BP. Shooting yourself in the foot: How immune cells induce antibiotic tolerance in microbial pathogens. *PLOS Pathogens.* 2021;17(7):e1009660. DOI: 10.1371/journal.ppat.1009660.
50. Bustos-Hamdan A, Bracho-Gallardo JI, Hamdan-Partida A, Bustos-Martínez J. Repositioning of Antibiotics in the Treatment of Viral Infections. *Current Microbiology.* 2024 Oct 26;81(12):427. DOI: 10.1007/s00284-024-03948-7.
51. Ruiz Ramos J, Salavert Lletí M. Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. *Revista Española de Quimioterapia*. 2019 May;32 Suppl 1(Suppl 1):45–54. PMID: 31131592; PMCID: PMC6555168.
52. Wright GD. Q&A: Antibiotic resistance: where does it come from and what can we do about it? *BMC Biology*. 2010;8:123. DOI: 10.1186/1741-7007-8-123.
53. Stallmach A, Steube A, Grunert P, et al. Fecal Microbiota Transfer. *Deutsches Ärzteblatt International*. 2020;117(3):31–38. DOI: 10.3238/arztebl.2020.0031.
54. Lin DM, Koskella B, Lin HC. Phage therapy: An alternative to antibiotics in the age of multi-drug resistance. *World Journal of Gastrointestinal Pharmacology and Therapeutics.* 2017;8(3):162–173. DOI: 10.4292/wjgpt.v8.i3.162.
55. Khomsiyah, Mohammad Zamroni, Adriano. Legal consequences of selling antibiotics drugs without doctor's prescription in pharmacies. *Journal Indonesia Law and Policy Review.* 2024;6(1):22–31. https://doi.org/10.56371/jirpl.v6i1.302.

56. Guizi Ye, Hongyu Wu, Wei Wang, Hong Xie, Yan Xu, Shizhen Fang, Jiang Zhong, Qingshan Huang. Antibacterial and synergistic effects of enzybiotics in combination with antimicrobial peptides and outer membrane permeabilizers against *Helicobacter pylori*. *FEMS Microbiology Letters*. 2025;372:fnaf130. https://doi.org/10.1093/femsle/fnaf130.
57. Gajic I, Tomic N, Lukovic B, Jovicevic M, Kekic D, Petrovic M, Jankovic M, Trudic A, Mitic Culafic D, Milenkovic M, et al. A Comprehensive Overview of Antibacterial Agents for Combating Multidrug-Resistant Bacteria: The Current Landscape, Development, Future Opportunities, and Challenges. *Antibiotics*. 2025;14(3):221. https://doi.org/10.3390/antibiotics14030221,
58. World Health Organization. Global action plan on antimicrobial resistance. WHO. 2021. https://www.emro.who.int/health-topics/drug-resistance/global-action-plan.html,
59. Ha DR, Haste NM, Gluckstein DP. The Role of Antibiotic Stewardship in Promoting Appropriate Antibiotic Use. *American Journal of Lifestyle Medicine.* 2017;13(4):376–383. DOI: 10.1177/1559827617700824

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Електронна адреса для листування khara_m@ukr.net