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MULTI-OMICS APPROACH TO THE STUDY OF MICROORGANISMS

In this review multi-omics (transcriptomic and proteomic) research approaches that have been widely implemented in modern microbiology are examined. The transcriptomic approach is important for predicting the resistance of microorganisms to specific antibiotics, as well as for understanding the mechanisms of the emergence of antibiotic resistance. In this review, the issues of studying the transcriptional response in microorganisms after in vitro exposure to subinhibitory concentrations of antimicrobial drugs are most extensively examined. It has been shown that antibiotics induce both phenotypic and genetic changes in bacterial cells, contributing to the emergence of resistance to them. Likewise, a proteomics-based approach broadens understanding of the bacterial strategy for antibiotic resistance, as well as improved understanding of the mechanisms by which antimicrobial resistance emerges, which will facilitate controlling of the growing epidemic of antibiotic-resistant infections in the future. In this review, the advantages of using one of the proteomics approaches widely used in clinical microbiology, MALDI-TOF MS, are considered more extensively. It has been shown that this approach is a more powerful tool for studying the protein profile in comparison with other methods.

Thus, the development of high-throughput transcriptomic and proteomic methods made analysis of large datasets of mRNA and proteins possible, which allows identifying functionally significant networks of intermolecular interactions, and thereby allowed to expand the modern understanding of mechanisms underlying the emergence of resistance to antimicrobial drugs.

Key words: transcriptomics, proteomics, subinhibitory concentration, antimicrobial drugs, microorganisms.

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Микроорганизмдерді зерттеудегі мультиомдық тәсіл

Бұл шолуда қазіргі микробиологияда кеңінен қолданылатын мультиомдық (транскриптомдық және протеомдық) зерттеу тәсілдері қарастырылған. Транскриптомдық тәсіл микроорганизмдердің белгілі бір антибиотиктерге төзімділігін болжау үшін, сондай-ақ антибиотикке төзімділіктің пайда болу механизмдерін түсіну үшін маңызды. Бұл шолуда микробқа қарсы препараттардың субингибиторлық концентрациясына in vitro әсер еткеннен кейін микроорганизмдердегі транскрипциялық реакцияны зерттеу мәселелері егжей-тегжейлі қарастырылады. Антибиотиктер бактериялық жасушаларда фенотиптік және генетикалық өзгерістерді тудырып, оларға төзімділіктің пайда болуына ықпал етеді. Сол сияқты, протеомикаға негізделген тәсіл, антибиотиктерге қарсы бактериялық стратегия идеясын кеңейтеді, сонымен қатар болашақта антибиотикке төзімді инфекциялардың өсіп келе жатқан эпидемиясын басқаруға мүмкіндік беретін микробқа қарсы тұрақтылықтың пайда болу механизмдерін толық түсінуге ықпал етеді. Бұл шолуда клиникалық микробиологияда кең таралған протеомика тәсілдерінің бірі – MALDI-TOF MS қолданудың артықшылықтары егжей-тегжейлі қарастырылады. Бұл тәсіл басқа әдістермен салыстырғанда ақуыз профилін зерттеудің ең қуатты құралы екендігі көрсетілген.

Осылайша, жоғары өнімді транскриптомика және протеомика әдістерінің дамуы мРНҚ мен ақуыздардың үлкен жиынтығын талдауға мүмкіндік берді, бұл молекулааралық өзара әрекеттесудің функционалды маңызды желілерін анықтауға мүмкіндік берді және осылайша микробқа қарсы тұрақтылықтың механизмдері туралы қазіргі идеяларды кеңейтуге мүмкіндік берді.

Түйін сөздер: транскриптомика, протеомика, субингибирлеуші концентрациялар, микробқа қарсы препараттар, микроорганизмдер.

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Мультиомный подход в изучении микроорганизмов

В данном обзоре рассмотрены мультиомные (транскриптомные и протеомные) подходы исследования, нашедшие широкое применение в современной микробиологии. Транскриптомный подход важен для прогнозирования устойчивости микроорганизмов к конкретным используемым антибиотикам, а также для понимания механизмов возникновения антибиотикоустойчивости. В этом обзоре наиболее подробно рассмотрены вопросы изучения транскрипционного ответа у микроорганизмов после воздействия на них in vitro субингибиторных концентраций антимикробных препаратов. Показано, что антибиотики вызывают в бактериальных клетках как фенотипические, так и генетические изменения, способствуя появлению резистентности к ним. Аналогично, подход, основанный на протеомике, расширяет представление о бактериальной стратегии противодействия антибиотикам, а также способствует более полному пониманию механизмов возникновения устойчивости к антимикробным препаратам, что позволит в будущем управлять растущей эпидемией устойчивых к антибиотикам инфекций. В настоящем обзоре наиболее подробно рассматриваются преимущества использования одного из широко распространённых в клинической микробиологии подходов протеомики, MALDI-TOF MS. Показано, что данный подход является наиболее мощным инструментом изучения белкового профиля по сравнению с другими методами.

Таким образом, развитие высокопроизводительных методов транскриптомики и протеомики сделало возможным анализ больших совокупностей мРНК и белков, что позволило выявить функционально значимые сети межмолекулярных взаимодействий и, тем самым, расширить современные представления о механизмах возникновения устойчивости к противомикробным препаратам.

Ключевые слова: транскриптомика, протеомика, субингибирующие концентрации, антимикробные препараты, микроорганизмы.

Abbreviations and symbols

DNA deoxyribonucleic acid; RNA ribonucleic acid; MIC – Minimum inhibitory Concentration; MALDI-TOF MS matrix-assisted laser desorption / ionization with time-of-flight separation; SNP single nucleotide polymorphism

Introduction

Currently, approaches to study biological objects are changing, ranging from the assessment of individual genes to the analysis of variability at the genome, transcriptome, and proteome levels, which became possible due to the use of high-resolution technologies with subsequent bioinformatic processing of the obtained array of multi-omics data [1]. It is the realm of -omics that has made the analysis of biological molecules cost-effective and highly productive [2].

Multi-omics approaches have found wide application in various fields of biology. Thus, -omics can contribute to advances in clinical microbiology. They contribute to a better understanding of microbial systems. Determination of transcriptomic and proteomic alterations in strains show different levels of resistance or different phenotypic responses to antibiotics [3].

A transcriptomic approach in the study of the mechanisms of bacterial resistance to antibiotics.

Transcriptomic analysis is an analysis of a complete set of transcripts produced by a cell under specific environmental conditions and is used to monitor the expression of bacterial genes in response to antimicrobial exposure. This type of analysis is widely used by many researchers. Initially, methods such as DNA chipping in combination with 2D polyacryamide gel electrophoresis were used for these purposes. For example, similar methods were implemented by Gmuender *et al.* [4], who studied the cellular response of *Haemophilus influenzae* after exposure to novobiocin and ciprofloxacin and found that the use of novobiocin affects the transcription of genes that depend on the DNA topology, and treatment with ciprofloxacin mainly increases expression of genes involved in DNA repair. Subsequently, the approach based on hybridization was replaced by a more promising technique, based on full sequencing of the RNA transcriptome (RNA-Seq). This method not only provides a deeper quantitative analysis of

gene expression in response to environmental signals, including exposure to antimicrobial drugs, but also allows to study the profile of noncoding RNAs. Also, using this approach, it was demonstrated that bacterial small nuclear RNAs (snRNAs) are involved in the post-transcriptional regulation of gene expression, and are also implicated in imparting antimicrobial resistance to antimicrobial drugs at various levels (such as the efflux of antimicrobial drugs, modification of the cell membrane, formation of biofilms, as well as DNA mutagenesis [5].

It should be noted that at present, the study of the cellular response of microorganisms after exposure to subinhibitory concentrations of antibiotics, which can cause not only low, but also high levels of resistance, is of great interest for researchers [6].

Phenotypic alterations caused by subinhibitory concentrations of antibiotics.

The effect of subinhibitory concentrations of antibiotics on the expression level of genes involved in major biological processes can lead to various phenotypic changes in microorganisms (biosynthetic and transport processes, metabolism of various compounds, bacterial responses to stress, etc.) [7].

Transcriptomic analysis of *Streptococcus pneumoniae* for a penicillin concentration equivalent to 0.5 of the MIC demonstrated that among 386 genes with altered transcription patterns, some genes are upregulated (for example, genes involved in the synthesis of the cell wall), and some of the genes, on the contrary, display a decrease in expression (for example, in genes encoding capsular polysaccharides) [8].

Moreover, the effect of subinhibitory antibiotic concentrations on the virulence of microorganisms is of great interest [7].

For example, the virulence of *P. aeruginosa* is enhanced by subinhibitory concentrations of tobramycin, tetracycline, norfloxacin [9]. Whereas, subinhibitory concentrations of azithromycin, ceftazidime, ciprofloxacin reduce the synthesis of virulence factors in the above microorganism [10].

de Freitas *et al.* studied morphological, biochemical, physiological changes, and virulence of *Bacteroides fragilis* after exposure to subinhibitory concentrations of ampicillin, ampicillin-sulbactam, clindamycin and chloramphenicol. It was found that the most noticeable morphological changes were caused by *β*-lactam drugs (ampicillin and ampicillin-sulbactam), these drugs caused bacterial filamentation (elongation) of bacterial cells. In this case, the normal morphology of all strains was restored after cultivation without the above-mentioned antimicrobial drugs. The authors note that among the biochemical characteristics, alterations affected carbohydrate fermentation. After treatment with antimicrobials, alterations in MIC (for ampicillin and ampicillin-sulbactam) were observed, which might be caused by selection of resistant strains or by selection of bacterial cells with altered physiological pathways and mutants' selection [11].

Genotypic alterations caused by subinhibitory concentrations of antibiotics.

Antibiotics, along with phenotypic changes in bacterial cells, cause genetic changes, contributing to the emergence and spread of resistance to them. The main changes caused by subinhibitory concentrations of antibiotics at the genotype level are the activation of horizontal gene transfer and an increase in the level of mutagenesis [7].

Thus, in *Bacteroides*, tetracycline induces the transfer of conjugative transposons carrying genes for resistance to tetracycline and erythromycin into recipient cells with their subsequent integration into the chromosome [12]. Another major genetic change caused by antibiotic subinhibitory concentrations is an increase in mutation rates. Thus, it is known that under the influence of subinhibitory concentrations of fluoroquinolones, a sharp increase in mutagenesis occurs in *Mycobacterium fortuitum* and *Streptococcus pneumoniae* [13, 14].

de Freitas *et al.* studied the transcriptional response of *Bacteroides fragilis* after *in vitro* exposure to subinhibitory concentration of metronidazole. As a result of this study, the authors identified 2146 genes encoding proteins, of which 1618 (77%) were attributed to Gene Ontology, i.e. they were associated with widely known cellular functions. Among the above 2,146 genes, 377 were common to all strains of *B. fragilis*, thus, are critical for the survival of bacteria. Activated or repressed genes were found that encode enzymes involved in several metabolic pathways and involved in the response to metronidazole exposure, such as drug activation, mechanisms of protection against superoxide ions, and high expression levels of efflux pumps and DNA repair genes [15].

Thus, during antibiotic therapy, microbial pathogens are often exposed to low concentrations of antibiotics. This creates conditions for an adaptive response that occurs at the transcriptome level and lead to increase of their virulence [7].

Proteomic approaches in the study of the mechanisms of bacterial resistance to antibiotics.

Proteomics is an indispensable tool for large-scale protein analysis and can be applied for understanding physiological alterations and for elucidation of mechanisms responsible for cellular processes in various genetic and environmental conditions. Thus, proteomics has expanded our knowledge of the mechanisms of bacterial antibiotic resistance. The recent development of a multidimensional approach combining proteomics with one or more -omics, including genomics, transcriptomics, and metabolomics, allows to better understand cellular physiology, metabolism at the system-wide level, including understanding the mechanisms of antibiotic resistance. As mentioned above, proteomic analysis is aimed at assessing the general profile of proteins in cells. This approach is used to qualitatively and quantitatively evaluate proteins expressed under certain conditions, including antimicrobial effects, and is also used to detect post-translational modification of proteins. Also, proteomic analysis allows to study the exoproteome, i.e. identification of all extracellular proteins that can either be freely secreted by the microorganism, or enclosed in extracellular vesicles. For example, Park *et al.* studied the cellular proteome and extracellular vesicles of *Pseudomonas aeruginosa*; they demonstrated that an increase in antibiotic resistance of biofilms is associated with modulation of both cellular and vesicular proteomes [16].

Most proteomic analyzes of antibiotic resistance can be divided into two large groups: comparison of resistant and susceptible bacteria and bacterial responses to the presence of antibiotics. Wherein, resistant strains can be clinical strains or strains obtained *in vitro*. In studies of the first type, most downregulated proteins are associated with secretion and metabolism, and most highly expressed are proteins involved in cell wall biogenesis, known mechanisms of resistance, metabolism, and transport of polysaccharides. In the second case, analysis of bacterial response to antibiotic exposure showed that the most frequently affected proteins are chaperone proteins and proteins involved in the stress response, amino acid metabolism, and energy metabolism. Some proteins involved in amino acid and energy metabolism are overexpressed, while others are underexpressed. The proteomic response is usually specific for each antibiotic, and, as described above, often involves proteins involved in energy and nitrogen metabolism, protein and nucleic acid synthesis, glucan biosynthesis and stress response. The results of proteomic studies are usually confirmed by genomic and / or transcriptomic analysis of strains [17].

Proteomic analyses for various bacteria after exposure to antibiotics (antibiotics, experimental conditions and methods of analysis are listed) are presented in the table 1.

According to the Table 1, Lata and Sharma compared the proteomic profiles of oflaxacin-susceptible and resistant clinical isolates of *M. tuberculosis* using 2-DE and MALDI-TOF-MS. Overexpression of 14 proteins was found in strains resistant to oflaxacin [19]. In the reference *E. coli* strain K12, about 4391 proteins have been identified so far. Ss can be seen from Table 1, the proteomic approach based on mass spectrometry made it possible to identify many proteins expressed in antibiotic-resistant E. coli strains [18, 20, 21].

The role of proteomic analysis in understanding the mechanisms of antimicrobial resistance manifestation.

In connection with the emergence of genes for antimicrobial resistance among pathogenic bacteria, proteomic analyzes have become crucial for assessing dynamic changes in protein expression at the systemic level. At the same time, it is of the greatest interest to obtain a quantitative picture of differentially expressed proteins under different conditions of therapy. The general mechanisms of antibiotic resistance are shown in Figure 1.

Thus, there are five ways for microorganisms to acquire antimicrobial resistance: 1) enzymatic modification of the antibiotic; 2) active elimination of the antibiotic from the microbial cell; 3) a change in the permeability of the outer membrane of the microbial cell, limiting the access of the antibiotic to the target sites; 4) acquisition of genes of the metabolic pathway alternative to that which is inhibited by the antibiotic [22]; 5) degradation of the antimicrobial agent; 6) modification of antibiotic targets, 7) overexpression of the target molecule [23].

One of the earliest proteomic studies in understanding the mechanism of resistance manifestation was the study of the resistance of *Pseudomonas aeruginosa* to ampicillin. At the same time, it was found that regulatory changes in gene expression, which entail a change in the composition of porins, can lead to a significant increase in the minimum inhibitory concentrations of antibiotics. It was also shown that the *Pseudomonas aeruginosa* strain has a low amount of porins in the outer membrane, which, together with the highly efficient operation of transmembrane pumps, makes this microorganism extremely resistant to a wide range of antibiotics [24, 25].

Table 1 – Proteomic analysis of antibiotic response in different bacteria [3]

Drug	Bacterial Strain	Primary MOA	Phenotypic Investigation, Target	Time of Exposure	Concentration	Proteomic Approach and Method	Proteome Coverage (%) *	Results, Selected DEPs	Reference
AMP CTX CFP	*E. coli*	Cell wall biosynthesis	Outer membrane vesicles, *β*-lactam resistance	12–84 h	AMP: 30 µg/mL CTX: 4 µg/mL CFP: 1.25 µg/mL	SDS-PAGE, MALDI-TOF-MS	1,639 (273 mapped) 260 (OMVs resistant) 270 (OMVs susceptible)	83 ↑, 49 ↓ (resistant)	[18]
OFX	*M. tuberculosis*	DNA gyrase	Planktonic, Mono-resistance	36 h	2 µg/mL (sub-MIC)	2-DE, MALDI-TOF MS	14	14 ↑	[19]
KAN	*E. coli*	Protein synthesis	Outer membrane Resistance	10 sequential subcultures	6.25 µg/mL (1/2 MIC)	2-DE, MALDI-TOF MS	11	6 ↑ 5 ↓	[20]
SM GEN CEF TET NA	*E. coli*	Protein synthesis, cell wall synthesis, DNA gyrase	Effect of low abundance of NarG and NarH on resistance	10 sequential subcultures	1/2 MIC	2-DE, MALDI-TOF MS	94	CAZ-R: 7↓ 6↑ SM-R: 5 ↓ 1↑ TET-R:7 ↓ 1↑ GEN-R: 9 ↓ 1↑ NA-R: 10 ↓	[21]

Note: *-Total number of proteins identified; AMP-Ampicillin; GEN-gentamicin; CEF- ceftazidime; OFX-ofloxacin; CTX – цефотаксим; CFP – цефоперазон; KAN – канамицин, TET – тетрациклин; NA – nalidixic acid; SM-streptomycin; DNA- deoxyribonucleic acid; MOA-Mode of action; arrow up-regulated and arrow down-downregulated proteins; MIC-minimum inhibitory concentration; OMV-outer membrane vesicles; SDS-PAGE- Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis; 2-DE – two-dimensional gel electrophoresis; MALDI-TOF MS – Matrix-assisted laser desorption ionization-time of flight mass spectrometry

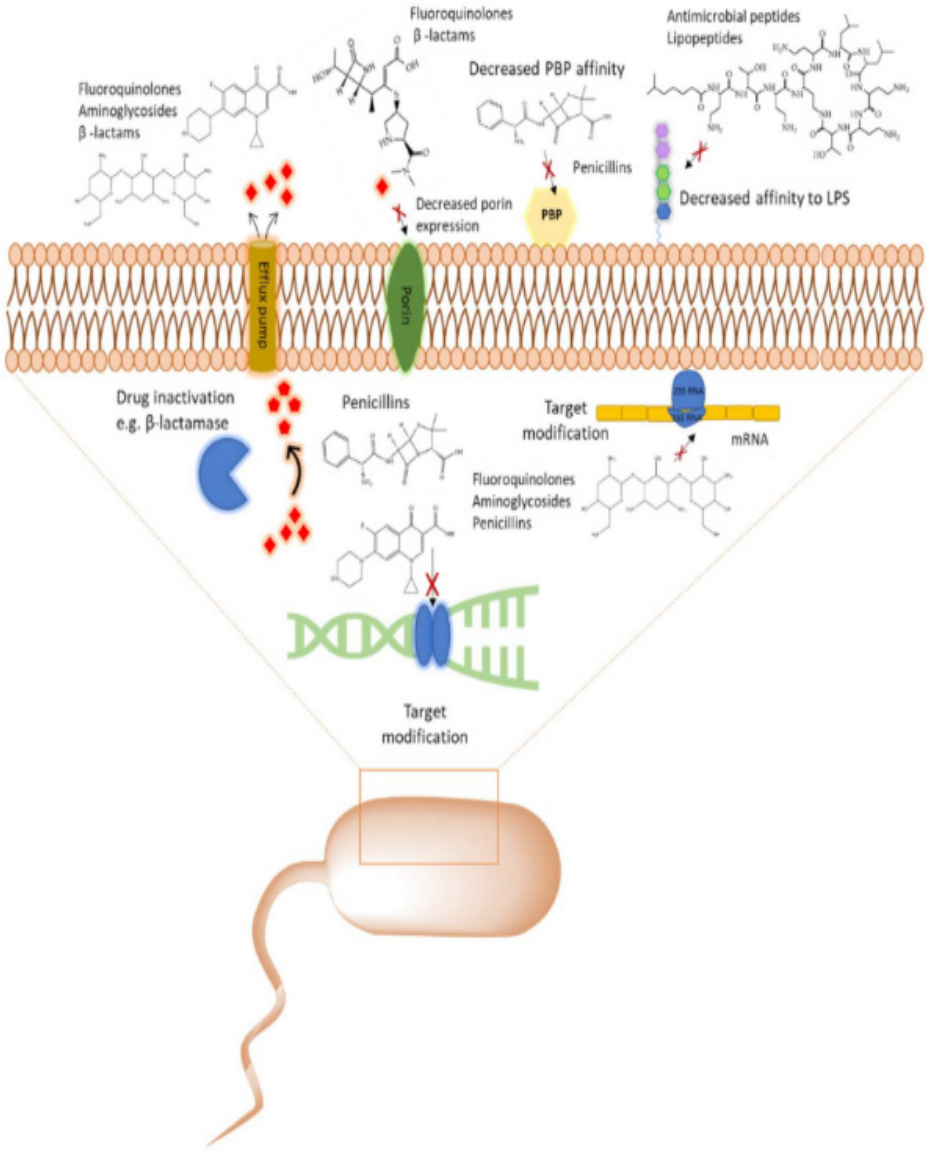

Figure 1 – Mechanisms of bacterial antibiotic resistance, which include target modification, drug inactivation, decreased affinity for lipopolysaccharides (LPS) and penicillin binding protein (PBP), and porin expression and pump efflux [3]

Currently, the most frequently studied using proteomic methods is the study of resistance to beta-lactam antibiotics, which account for more than half of all used antimicrobial drugs [17]. Beta-lactam antibiotics (penicillin, cephalosporin, carbapenens, monobactam and beta-lactamase inhibitors) disrupt the synthesis and / or stability of the cell membrane, thereby disrupting the biogenesis of the cell wall and lead to a loss of selective permeability and osmotic permeability, which ultimately leads to death of a bacterial cell. The main mechanism of resistance to beta-lactam antibiotics is the presence of proteins that hydrolyze antibiotics, known as beta-lactamases [26, 27].

Other important mechanisms include imbalances in transport proteins such as efflux pumps and porins and altered targets for penicillin binding proteins that reduce the affinity of β-lactams (by binding to the active serine site of penicillin binding proteins, resulting in inactive forms of enzymes that cannot catalyze both synthesis and cross-linking of peptidoglycan, which is important for achieving a rigid cell structure) [3, 22, 28]. Thus, the C-terminal domains of all penicillin-binding proteins are targets for β-lactam antibiotics. These antibiotics contain a β-lactam ring, a structural analogue of the D-Ala-D-Ala dipeptide, and therefore act as competitive inhibitors of penicillin-binding proteins (Figure 2).

The interaction between the carbonyl group in the β-lactam ring and the hydroxyl group of serine in the active site of the penicillin-binding protein leads to the formation of an inactive acylated form of the enzyme. Irreversible inhibition disrupts the synthesis of the bacterial cell wall [29].

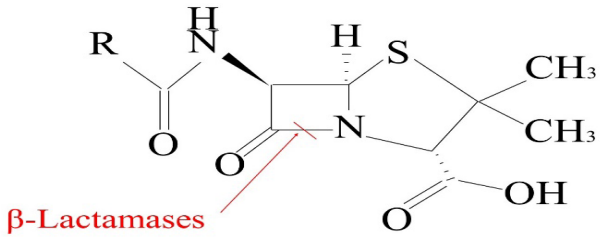

Figure 2 – Structure of β-lactams [29]

β-lactamases are a superfamily of enzymes, which today has more than 2000 representatives. It is interesting to note that the evolution of *β*-lactamases occurs according to two main mechanisms: the appearance of new mutations in the genes of known enzymes and the appearance of enzymes with a new structure. The high frequency of *β*-lactamase mutations and the localization of their genes on mobile genetic elements contribute to the rapid spread of resistant bacteria, which is currently a global threat. In general, the question of the origin of bacterial enzymes responsible for the development of resistance during evolution remains controversial. Enzymes, which perform vital functions and are responsible for the biosynthesis of cell wall polysaccharides, proteins, nucleic acids, and metabolites, serve as targets for antibiotics. Interestingly, modification of the active sites of target enzymes promoted their ability to use antibiotics as substrates [29].

Subsequent proteomic studies addressing the manifestation of metronidazole resistance in resistant *B. fragilis* ATCC 25285 showed that proteomic changes affected a wide range of metabolic proteins, including lactate dehydrogenase (upregulation) and flavodoxin (downregulation), which may be involved in electron transfer reactions: disruption of enzymatic activity of the pyruvate-ferredoxin oxidoreductase (PorA) complex [30].

Thus, it should be noted that resistance to a single antibiotic can be determined by several different enzymes and mechanisms. Moreover, quite often even one cell has different mechanisms of resistance to the same antibiotic.

Proteomic methods for the detection of antimicrobial resistance.

Antibiotic resistance mechanisms do provide microbes with the ability to bypass the effects of antibiotics and survive after exposure. Proteomics has emerged as an important tool in this area of research [22].

Proteomic methods are constantly evolving, and a wide variety of such methods and applications are currently available. One of the earliest methods successfully implemented in microbiology was the gel method (2D-PAGE). This method allowed the creation of maps of proteomes, thus giving a detailed view of the general expression of bacterial genes under certain conditions. For this, bacteria are grown *in vitro* under strictly controlled conditions, and the obtained comparative studies were used to identify a protein that correlates with its resistance [22]. Then the gel-free method became more popular, and the gel method was replaced by methods of quantitative analysis – metabolic and chemical labeling. Subsequent advances in the development of high-throughput and automated mass spectrometry instruments (from liquid chromatography-LC-MS to MALDI-TOF MS) have facilitated the application of quantitative proteomics using label-free strategies. The increased sensitivity of mass spectrometers, together with improved technologies for sample preparation and protein fractionation, have allowed for a more complete study of proteomes. In this respect, quantitative proteomics based on mass spectrometry is the most powerful tool for studying the protein profile compared with other methods [5].

Thus, MALDI-TOF MS applications for the detection of antimicrobial resistance can be divided, depending on the type of target and methodology, into the following approaches [27].

1. *Identification of the entire cell profile.* It includes the identification of differences in the spectra of all proteins from susceptible and resistant strains. Similar results were obtained for the important gram-negative anaerobic pathogen, *B. fragilis*. Thus, the use of MALDI-TOF MS identified two groups of bacteroides (I and II). Group II, carrying the *cfiA* gene, encodes a powerful metallo-beta-lactamase, and group I, which does not have it, differ in specific peaks in the spectra of their profile [31, 32].

In our work, the use of MALDI-TOF / MS also identify the bacteroid strain BFR_KZ01, isolated from a patient with peritonitis, belonging to group II (cfA-positive), but still susceptible to meropenem, due to the presence of a gene in a "silent state" [33].

2. *Identification of antibiotic and hydrolysis product*. This approach has been reported for important clinical carbapenemases and extended-release beta-lactamases. Antibiotics and degradation products are usually analyzed in the mass range from 100 to 1000 Da. During the ionization process, matrix usually protonates the antibiotic, increasing the mass of the antibiotic. During the hydrolysis of lactams, some groups of molecules are lost. As newly hydrolyzed molecules become unstable due to the breaking of lactam rings, and tend to break into different fragments, compounds with different molecular weights are formed. The masses of such peaks are unique for each antibiotic and can be used for the detection of a specific antibiotic [34]. Strains negative for beta-lactamase do not change the molecular weight of beta-lactam [35]. In addition, the procedure allows for quantitative analysis, which is useful for direct comparison with MIC values, and provides excellent resolution. In addition, this method can be improved by using beta-lactamase inhibitors for identification of specific types of beta-lactamases. This method has been used for resistance to carbapenemase, chemicals with metallo-beta-lactamase encoded by *cifA*, in blood samples [36].

3. *Detection of proteins that confer resistance to the microorganism*. In this case, MALDI-TOF MS can establish some microbial biomarkers (mainly proteins or their fragments obtained after cleavage by trypsin), which confer resistance to the pathogen. For example, methicillin-resistant *S. aureus,* positive for agr (additional gene regulator) and carrying the mec class A complex, was identified by detecting a small peptide called PSM-mec in whole cells [37].

4. *Analysis of the cell wall*. The cell wall is a target for antibiotics and a barrier to other antibiotics, which act in the cytosol. For distinguishing various resistant and susceptible strains of gram-negative bacteria, specific components of the outer membrane such as porins, efflux pumps and lipopolysaccharides were quantitatively identified using MALDI-TOF MS methods [38]. For example, changes in the lipopolysaccharide lipid A structure that occur during the appearance of colistin resistance in *A. baumanni* can also be detected using MALDI-TOF MS [39].

5. *Discovery of mutations in resistance genes by sequencing.* MALDI-TOF MS methods were used for DNA sequencing analysis. Thereby, Pusch *et al* applied SNP genotyping based on MALDI-TOF MS in their study [40]. However, this approach is time consuming and does not offer any advantages over standard sequencing protocols.

6. Marking of stable isotopes and monitoring cell growth. The technology of labeling stable isotopes with amino acids in cell culture with the same isotope was used to distinguish between resistant and susceptible *P. aeruginosa* strains to meropenem, tobramycin, and ciprofloxacin [41].

Sensitive and resistant bacteria of the same species differ in growth in the presence of a particular antibiotic. For example, Lange *et al.* developed an antibiotic-sensitive-rapid test based on measuring the number of peptides and proteins within the range of spectra. These quantities correlate with the number of microorganisms and therefore with the growth of the microorganism. [33, 42].

It should be noted that most of the expressed proteins represent a stable phenotype and most of the different changes observed in resistant strains are not metabolic disorders in bacteria. Therefore, proteomics, together with other high-throughput approaches, can help understand metabolic pathways and their impact on antibiotic resistance [22].

Thus, proteomics complements comparative genomics and transcriptomic profiles by providing data on the nature of proteins. It provides information, which is unavailable for other methods, for example, in the event of post-translational modifications, subcellular protein localization, and others [22].

Conclusion

Today, despite the enormous contribution of antibiotics to human health, one of the most alarming consequences of antibiotics overuse is the emergence and spread of resistant microorganisms [43]. Addressing the challenges of antibiotic resistance requires in-depth understanding of the mechanisms by which resistance emerges. The coordinated use of various approaches, including genomics, transcriptomics, together with good standard proteomic methods, is intended to improve the ability to detect bacterial resistance, understand the mechanisms of resistance and the response of virulence in microorganisms [27].

During antibiotic therapy, pathogens are often exposed to low concentrations of antimicrobial drugs, which creates conditions for an adaptive response that occurs at the level transcriptome and is manifested by an increase in their virulence. Subinhibitory concentrations of antibiotics affect expression levels of genes involved in major biological processes and can lead to various genotypic and phenotypic changes in microorganisms. The study of the mechanisms of bacterial response to subin-

hibitory concentrations of antibiotics allows to propose fundamentally new ways to combat pathogenic microorganisms, as well as to search for substances that specifically act on systems for controlling pathogenic properties [7].

MALDI-TOF mass spectrometry is one of the highly efficient, accurate and at the same time low-cost proteomic methods, which has become widespread in clinical microbiology in recent years. The emergence of new applications of MALDI-TOF mass spectrometry allows to improve the diagnosis of infections and determine the resistance of pathogens to antibiotics, what makes this technique especially attractive for multidisciplinary hospitals [44].

Thus, -omic technologies are designed to improve the current understanding of microbial biology. Highly productive multi-omics methods open new possibilities for a larger-scale analysis of mRNA and protein expression. The results of proteomic and transcriptomic analysis, processed by bioinformatics methods, provide a powerful basis for understanding the functional significance of transcripts and proteins of microorganisms under normal conditions and under stress conditions. Comparative multiomics data are also intended to facilitate understanding of phenotypic differences in bacteria (level of drug susceptibility as well as virulence).

It should be noted that in Kazakhstan, to date, studies of microorganisms based on a multi-omics approach including transcriptomic and proteomic analysis has not been undertaken. In this connection, the use of the above approaches aimed at obtaining new data on the mechanisms of resistance of microorganisms will be generally relevant for the fundamental science.

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Conflict of interest

All authors are familiar with information provided in the article and declare no conflicts of interest.

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