ISSN 2075-5457 (print), ISSN 2220-9697 (online)

DOI: 10.26565/2075-5457-2025-44-3
UDC: 619:614.31:631.57+ 579.253.4

Evolution of metallo-beta-lactamases in focus of the antibiotic resistance problem

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The article considers the place of metallo-beta-lactamases among the mechanisms of antibiotic resistance. These enzymes have important differences from serine beta-lactamases, which affect the mechanism of their catalytic activity, inhibition, rate of spread and evolution. The history of the study and the social significance of antibiotic resistance in general and beta-lactamases in particular are described. The classifications of enzymes that hydrolyze the beta-lactam ring are presented, the structure of the reaction center of metallo-beta-lactamases and a hypothetical model of its functioning are described. Two fundamentally different mechanisms of inhibition of metallo-beta-lactamases (zinc-dependent and zinc-independent) and their consequences for the development of therapeutic strategies are also considered. Metallo-beta-lactamases were distributed among non-pathogenic natural populations of bacteria, and then began to spread to pathogenic ones (initially gram-negative), which determines the importance of their study from the point of view of public health. The high rate of spread of these enzymes is due to their localization in structures such as integrons, insertion sequences and conjugation plasmids and can be illustrated by the example of the NDM enzyme, first isolated in New Delhi in 2009, which spread throughout the world in ten years and formed three dozen mutant variants. The fact that metallo-beta-lactamases arose in evolution twice is almost proven. Today, the phylogenetic relationships between different representatives of this enzyme family have been more precisely clarified and the presence of ten monophyletic groups has been established. The data presented in the article can serve as a starting point for planning comprehensive work on predicting the evolution of metallo-beta-lactamases, which carry serious risks for the treatment of infectious diseases.

Keywords: *mechanisms of antibiotic resistance, molecular evolution of bacteria, molecular adaptations*

Cite this article: *Moskalov V.B., Kadenko I.V., Mukhin A.M. (2025). Evolution of metallo-beta-lactamases in focus of the antibiotic resistance problem. The Journal of V. N. Karazin Kharkiv National University. Series Biology, 44, p. 23-31. https://doi.org/10.26565/2075-5457-2025-44-3*

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Received: 10.03.2025 / Revised: 22.04.2025 / Accepted: 26.05.2025

Introduction

Antibiotics have played a major role in reducing human mortality from bacterial infections and have continued to save countless lives every day for more than 75 years since the start of their commercial use. Today, antibiotics are widely used to treat a wide range of infectious diseases, including urinary tract infections, skin and soft tissue infections, pneumonia, endocarditis, meningitis, and sepsis, as well as they are necessary for routine and complex medical procedures, including surgery (Banin, 2017). It is obvious that the success of medicine and the maintenance of public health depend on the effectiveness of antibiotics. However, almost immediately after the advent of antibiotics, the phenomenon of antibiotic resistance arose, calling into question the success of antibiotic therapy. The development of new drugs has been a key strategy for breaking through antibiotic resistance. However, as the mechanisms of antibiotic resistance development accelerate and the entry of new antibiotics into the market slows, this strategy is becoming less effective (Banin, 2017). A recent UK government-funded O'Neill report estimated that by 2050, around 10 million people a year will die from antibiotic-resistant infections (O'Neill, 2014). This requires a detailed analysis of the resistance molecular aspects and the issues of their evolution and spread to develop new strategies for anti-infective medicine.

The molecular mechanisms of antibiotic resistance in bacteria are quite diverse, but they can be divided into three main groups: (I) preventing contact of the cell contents with the antibiotic; (II) modification of targets; and (III) inactivation or destruction of the antibiotic molecule (Church, 2021; Urban-Chmiel, 2021; Lin, 2015). The first group of mechanisms includes in particular the biofilm formation as well as the efflux pumps work. The biofilm is a surface-attached community of bacterial cells that forms a protective

extracellular polymer matrix around themselves, which reduces the diffusion of antimicrobial molecules. The efflux pumps remove antibiotic molecules from the periplasm of gram-negative bacteria or the cytoplasm of gram-positive bacteria, preventing the achievement of an effective concentration of this substance in the cell (Banin, 2017; Church, 2021; Urban-Chmiel, 2021; Frieri, 2017). Among the mechanisms of the second group, it is worth noting the protection of the target by additional molecules, non-genetically induced modification of the target site, mutations of the target site, enzymatic changes in target sites, and complete replacement or bypass of the target in metabolic pathways (Banin, 2017; MacGowan, 2017; Church, 2021; Urban-Chmiel, 2021; Frieri, 2017). In this article, we will focus on one of the mechanisms of the third group, which involves the inactivation or destruction of the antibiotic molecule, on beta-lactamases, namely, metallo-beta-lactamases (MacGowan, 2017; Church, 2021; Urban-Chmiel, 2021).

The study of the spread of antibiotic resistance among bacteria potentially pathogenic to humans began with the discovery of the role of uncontrolled use of antibiotics in animal husbandry (Frieri, 2017; Podolsky, 2018; Church, 2021). Then, an increasing prevalence of "gram-negative" infections was established in hospitalized patients. This is probably due to the more efficient operation of their efflux pumps. The discovery in the 1960s of a plasmid-mediated resistance to multiple antibacterial agents led to the concept of "superbug". The term "superbug" was coined by John Osmundsen in 1966 while analyzing Anderson's work on plasmid-mediated bacterial resistance to several antibacterial agents, who was concerned about the risk of the release into the environment of resistant bacteria created by artificial selection in the laboratory (Osmundsen, 1966; Podolsky, 2018). At the same time, the question about the risk of an epidemic of drug resistance among microbes was raised around the world, i.e. multiple drug resistance was a global problem for humanity. In the 1980s, Stuart Levy and his colleagues summarized the factors contributing the spread of antibiotic resistance known at that time, highlighting: the use of antibiotics without a prescription, as growth promoters in agribusiness, in cases where they are ineffective or not needed (e.g., infectious diseases caused by viruses, before the bacterial component is involved), and marketing strategies for antibiotics as “miracle drugs” (Levy, 1981; Podolsky, 2018). This made it possible to propose measures to reduce the intensity of the antibiotic resistance spread, including: training of medical personnel, veterinarians and agricultural workers in the rational use of antimicrobials, monitoring the use of antimicrobials and traces of their use in agricultural products, and surveillance of new resistant organisms. At the same time, the slowdown in the rate at which new antimicrobial drugs are brought to market is leading to a lag in the race between new superbugs and effective antibiotics. Today, an important aspect of studying the evolution of antibiotic resistance is the context of global climate change (Podolsky, 2018).

The high rate of antibiotic resistance spread in bacterial populations is associated with both the intense pressure of antibiotics as a selection factor and the significant rate of horizontal gene transfer by conjugation of plasmids (*Enterococcus spp., Staphylococcus spp., Streptococcus spp*.) and transposons (*Listeria*), transduction and transformation in these populations (Urban-Chmiel, 2021; Sabtu, 2015). The efficiency and speed of the resistant phenotype spread to populations of previously susceptible species are difficult to predict today. Thus, the beta-lactamase gene from staphylococci (e.g. *blaZ*) spreads very successfully in populations of *Staphylococcus aureus, Haemophilus influenzae* and many bacteria of the Enterobacteriaceae family, but is not found among enterococci (Charpentier, 1999; Olsen, 2006). At the same time, enterococci`s genes of resistance to vancomycin (such as *vanA*) are not found in populations of *Staphylococcus aureus*. (Charpentier, 1999). The facts described indicate the importance of a comprehensive assessment of the change and spread of individual antibiotic resistance mechanisms.

Among the wide range of molecular mechanisms of antibiotic resistance, metallo-beta-lactamases are perhaps the most alarming. This is due to a number of reasons: a unique mechanism of catalytic activity, multiple drug resistance, and a high rate of spread (Boyd, 2020). In view of the above circumstances, a generalization of the available information on the evolution of metallo-beta-lactamases seems relevant.

The article analyzes the variability of metallo-beta-lactamases, their consequences for catalytic activity and the outcome of interactions with inhibitors, as well as the spread of new gene variants among bacterial populations and possible directions of further evolution.

Molecular variability, catalysis and inhibition of metallo-beta-lactamases

Beta-lactamases belong to group III of antibiotic resistance mechanisms, since they catalyze the destruction of the beta-lactam ring and, as a result, the inactivation of beta-lactam antibiotics. These enzymes have become known shortly after the first use of antibiotics with the discovery of penicillinase (beta-lactamase) in 1940 (Munita, 2016; Blair, 2015). To date, extended-spectrum beta-lactamases (ESBLs) that exhibit activity against the new generation of antibiotics such as oxyiminocephalosporins have been identified. The spread

of diverse ESBLs and carbapenemases, oxacillinases (OXA) and NDM enzymes in Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* leads to the emergence of isolates resistant to all beta-lactam antibiotics, which has serious implications for the treatment of severe infections and poses a threat to public health. Genes encoding ESBLs have been disseminated by gene escape from the chromosome of soil bacteria *Kluyvera spp*. of Enterobacteriaceae family, related to *Escherichia coli* (Munita, 2016; Blair, 2015). This escape has been facilitated by insertional sequences, particularly ISEcp1, with subsequent transfer by conjugative plasmids to many bacterial species. For example, the $bla^{CTX\text{-}M14}$ gene is frequently associated with the IncK pCT plasmid in human, animal, and environmental isolates of bacteria (Blair, 2015).

Today, there are many classifications of beta-lactamases, but the main one is revised Ambler classification, according to which metallo-beta-lactamases belong to class B (last column in Fig. 1) in contrast to classes A, C and D, which include serine beta-lactamases (Ambler, 1980; Hall, 2005). Metallo-beta-lactamases are inhibited by chelating agents such as ethylenediaminetetraacetic acid, but not serine lactamase inhibitors such as clavulanic acid, sulbactam, or tazobactam. They are also not inhibited by NXL104, which inhibits class A and C enzymes (Munita, 2016; Palzkill, 2013).

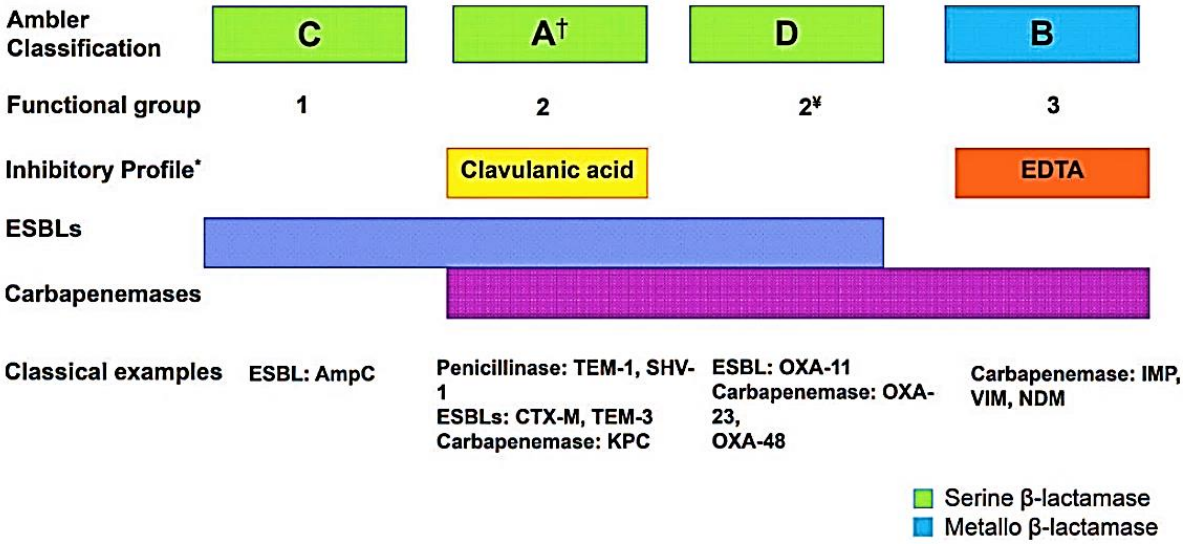

Fig. 1. Common classifications of beta-lactamases (Munita, 2016)

Class B beta-lactamases, or metallo-beta-lactamases, require zinc ions to catalyze the hydrolysis of beta-lactams and have no structural homology to serine beta-lactamases of other classes. Class B enzymes can be divided into three subclasses B_1, B_2, and B_3 based on their amino acid sequences, substrate profile, and metal ion-binding amino acids (Page, 2008). Table 1 shows the amino acid residues that are part of the reaction centers of various subclasses of these enzymes and ensure the work of zinc ions.

Table 1. Amino acid surpluses that participate in the coordination of zinc (Page, 2008)

Subclass	First ion Zn^{2+}			Second ion Zn^{2+}		
B_1	His116	His118	His196	Asp120	Cys221	His263
B_2	Asn116	His118	His196	Asp120	Cys221	His263
B_3	His/Gln116	His118	His196	Asp120	His121	His263

Note. His – Histidine, Asn – Asparagine, Gln – Glutamine, Asp – Aspartic acid, Cys – Cysteine

The role of zinc ions in the mechanism of catalysis has not been fully established. It is likely similar to zinc peptidases such as carboxypeptidase A and thermolysin: after substrate binding, a zinc-bound water molecule, deprotonated by the Asp120 residue, attacks the carbonyl center with the formation of a negatively charged tetrahedral intermediate, which is stabilized by its interaction with the metal ion. Then the Asp120 residue donates a proton to the nitrogen, which ensures the cleavage of the C–N bond with subsequent cleavage of the product from the active site of the enzyme. The main drawback of this model is the stage of

ISSN 2075-5457 (print), ISSN 2220-9697 (online)

removing the proton from the water bound to the zinc, since it is already ionized (Palzkill, 2013; Walsh, 2005; Page, 2008; Bebrone, 2007). The active sites of zinc-ion enzymes (marked by dark blue spheres) of the three class B subclasses are shown in Fig. 2. As we can see in the figure, enzymes of subclasses B_1 and B_3 usually contain two zinc ions in their composition, and subclass B_2 contains only one. Amino acids 120, 263 are obligatory, and amino acids 221 and 121 are optional, in coordinating the zinc ion, which is common to all forms of the enzyme. The second zinc atom in both forms of the enzyme for which it is characteristic is coordinated by amino acids at positions 118, 116 and 196. Hence, the amino acids in the described positions participate in the formation of the reaction center of metallo-beta-lactamases.

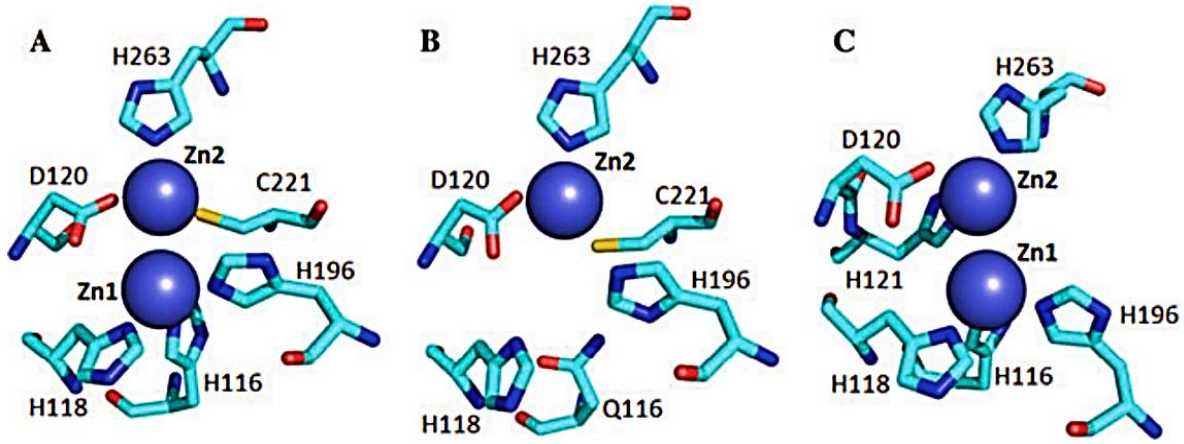

Fig. 2. Active sites of metallo-beta-lactamases (Palzkill, 2013): a – active site residues of the subclass B_1 enzyme CcrA with a zinc chelation site (*Bacteroides fragilis*); b – active site residues of the subclass B_2 monozinc enzyme with a zinc chelation site (*Aeromonas hydrophila*); c – active site residues of the subclass B_3 enzyme L1 with a zinc chelation site (*Stenotrophomonas maltophilia*)

Metallo-beta-lactamases are apparently inhibited by chelating agents such as ethylenediaminetetraacetic acid (EDTA), which absorb metal ions. However, it has now been established that there are two strategies for achieving inhibition: Zn^{2+}-independent and Zn^{2+}-dependent (Fig. 3). The diversity of amino acids and the lack of a deep active site pocket are obstacles to the Zn^{2+}-independent approach. On the other hand, Zn^{2+}-dependent inhibitors may bind non-target proteins, since metal-dependent enzymes are very common in the human body, and divalent cations such as Ca^{2+} and Mg^{2+} circulating in plasma and tissues can compete with Zn^{2+} for inhibitor molecules (Ju, 2018; Rotondo, 2017; Mojica, 2022).

The Zn^{2+}-dependent inhibition mechanism (Ju, 2018) involves the binding of metal ions and the formation of ternary complexes [enzyme–Zn^{2+}–inhibitor] such as 1,2,4-triazole-3-thiones, captopril and analogues, cyclic boronates, bisthiazolidines, mercaptoacetate, mercaptocarboxylate, mercaptophosphonates, aminophthalic acid derivatives, disubstituted succinic acids, etc. (Fig. 3 A1), or the removal of the metal and the formation of a metal–inhibitor complex with EDTA, 1,10-phenanthroline, picolinic acid and enzyme with a reduced amount of bound Zn^{2+} (Fig. 3 A2).

Zn^{2+}-independent inactivation of metallo-beta-lactamases (Ju, 2018) can be carried out through covalent modification of the enzyme molecule (mercaptophenylacetic acid, moxalactam, cephamycin, etc., Fig. 3 B), allosteric regulation, i.e. binding to the allosteric center (arginine peptides, graphene oxide and nanotubes, nanochains and single-stranded DNA, Fig. 3 C) and through unknown mechanisms (azolylthioacetamides, several different classes of thionyl peptides, hydroxamates and “reverse” hydroxamates, maleic acids, mitoxantrone, triazoles, mercaptotriazoles, etc.).

As we can see, the mechanisms of inhibition of metallo-beta-lactamases are quite unique and require a balanced approach in drug development, since they can affect proteins of the human body containing divalent metals.

Distribution and evolution of metallo-beta-lactamases

Metallo-beta-lactamases were initially not considered a serious problem for antibiotic therapy because they were found in the chromosomes of only non-pathogenic bacteria. However, in the 1990s,

ISSN 2075-5457 (print), ISSN 2220-9697 (online)

metallo-beta-lactamases of the IMP and VIM types were discovered (Palzkill, 2013; Walsh, 2005). They were encoded as gene cassettes in integron structures and were associated with transposons, so they can be integrated into the bacterial chromosome or plasmid, which may ensure their rapid horizontal transfer between different bacterial species (Palzkill, 2013; Walsh, 2005, Page, 2008; Bebrone, 2007). This

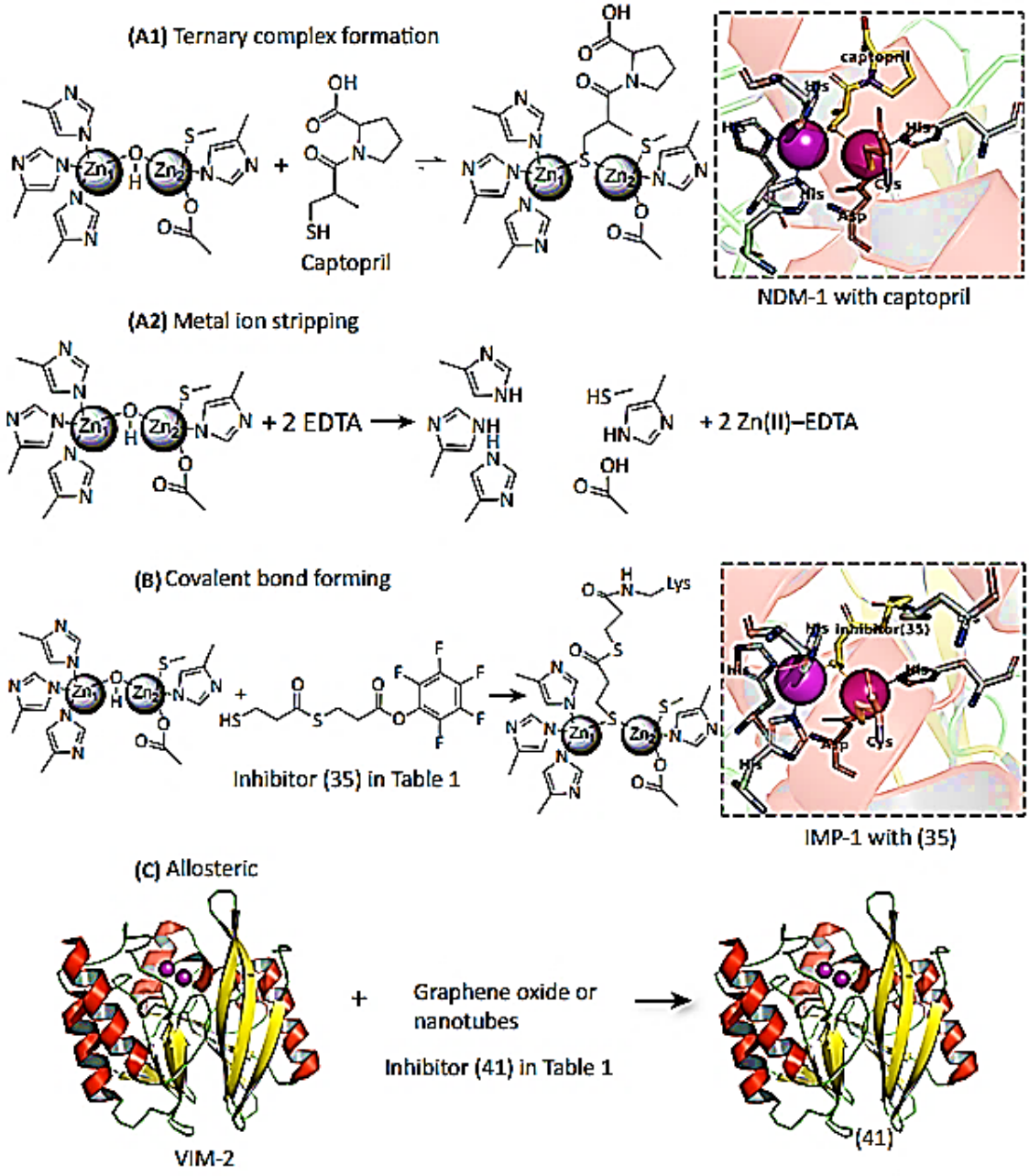

Fig. 3. Mechanisms of metallo-beta-lactamases inhibition (Ju, 2018). A1 – inhibiting by formation of ternary complexes [enzyme–Zn^{2+}–inhibitor]; A2 – inhibiting by zinc ion(s) removing; B – inhibiting through covalent modification of the enzyme molecule; C – inhibiting through allosteric regulation

assumption was later confirmed in practice: a widespread distribution of enzymes of this class was observed among the family of gram-negative bacteria Enterobacteriaceae, which includes such well-known pathogens as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Palzkill, 2013; Walsh, 2005; Bebrone, 2007; Page, 2008). Thus, NDM-1-lactamase was first identified in 2008 in *Klebsiella pneumoniae* and *Escherichia coli* in a patient returning to Sweden from India, where this enzyme was found with significant frequency among Enterobacteriaceae (Mojica, 2022). The *bla*$^{NDM-1}$ gene has been found in several types of

ISSN 2075-5457 (print), ISSN 2220-9697 (online)

plasmids, including IncA/C, IncF, IncL/M, and was capable of being transferred among gram-negative bacteria by conjugation. However, unlike the situation with the genes encoding the metallo-beta-lactamases IMP and VIM, the *bla*$^{NDM-1}$ gene was not found in integron structures, and its rapid spread may be explained by association with insertion sequences (Palzkill, 2013). The presence of metallo-beta-lactamase genes in insertion sequences and integrons, their integration into conjugation plasmids provide opportunities for their rapid spread. This distribution is observed primarily among related gram-negative bacteria, but gradually this mechanism will most likely begin to be transmitted to distant groups of bacteria, including pathogenic ones.

The origin of metallo-beta-lactamases is not entirely clear, but there is some information that partially sheds light on this problem. The presence of homologues of metallo-beta-lactamase genes in all domains of Eubacteria, Archaebacteria and Eukaryota indicates a very ancient origin of this family. In particular, the homology of these enzymes with the human *Gox* gene (hydroxyacylglutathione hydrolase, glyoxalase II) may be evidence of this (Garau, 2005). An important aspect of elucidating the origin of metallo-beta-lactamases is the function of the parent protein from which they are derived. Among the family of these proteins (Diene, 2023) there are enzymes with beta-lactamase, nuclease, ribonuclease, lactonase, glyoxalase, phytase and potentially other unidentified hydrolase activities. Also noteworthy are groups of proteins with non-enzymatic activity, namely, molecules involved in the regulation of osmosis, quorum sensing, and transport of substances across the membrane.

The evolution of metallo-beta-lactamases begins with the accumulation of mutations in different positions of their structure and undergoes different evolutionary trajectories with the appearance of different signs. These signs can always be described on the basis of tracking the purified proteins. The development of the evolution of these enzymes in a manner that is as close as possible to the native one implies: (1) the development of the resistance phenotype in model systems of various bacteria; (2) expression of the enzyme in the body; (3) a comprehensive assessment of the level of expression of the active protein in the periplasm, either in a separate form or in a membrane-bound form; (4) investigation of the effect of Zn^{2+} deprivation on the resistance phenotype and kinetic stability of the apoenzyme; (5) the relationship of these data with *in vitro* studies of catalytic efficiency and affinity for zinc binding; and (6) the importance of kinetic parameters in periplasmic extracts or spheroplasts to bridge the gap between *in vitro* and cell experiments. In addition, the understanding of the folding of metallo-beta-lactamases associated with metal-binding in the periplasm highlights the need for new experimental and computational tools, especially quiet ones that measure different time scales (López, 2022). As we can see, not all the tools for determining the origin and evolution of metallo-beta-lactamases have been developed yet, but we can evaluate some evolutionary processes, since they have been taking place in recent decades literally before our eyes.

Some metallo-beta-lactamases have become widespread quite recently, and we can observe the localization of their emergence and waves of spread. For example, NDM-1 was discovered in 2007 in a clinical isolate of *Klebsiella pneumoniae* from a urine culture of a 59-year-old male patient who was hospitalized in Ludhiana and New Delhi, and the name of the enzyme is based on the capital of India (Wei, 2015). Soon, NDM-1 began to be detected throughout the Indian subcontinent, as well as in people who had contact with its inhabitants – Great Britain (Wei, 2015; Mojica, 2022). The evolution and spread of the NDM-1 enzyme with carbapenemase activity is shown in Fig. 4. As we can see in the figure, over the ten years of observation since the first detection of this enzyme in a bacterial isolate from a patient, three dozen of its variants have emerged, containing from one to five non-synonymous mutations. There is also a worldwide distribution of NDM.

As we noted above, metallo-beta-lactamases can be divided into three subgroups by their structure – B_1, B_2 and B_3. At present, it is considered an established fact that the first two of them are homologous to each other, and the third forms a separate group in the phylogenetic sense. This fact indicates that these enzymes arose in evolution twice. Some models assume a single origin of metallo-beta-lactamases (about 5% of existing models), which are intriguing but unconvincing (Garau, 2005; Alderson, 2014).

The latest phylogenetic tree, constructed from 2290 unique metallo-beta-lactamase sequences identified using genomic and metagenomic screening data, provides a more complete picture of the evolution of this important class of antibiotic resistance genes (Berglund, 2021). Genes from subclades B_1 and B_3 are structured into five and four monophyletic groups, respectively, largely reflecting their host taxonomy, biochemical characteristics, and evolutionary history.

ISSN 2075-5457 (print), ISSN 2220-9697 (online)

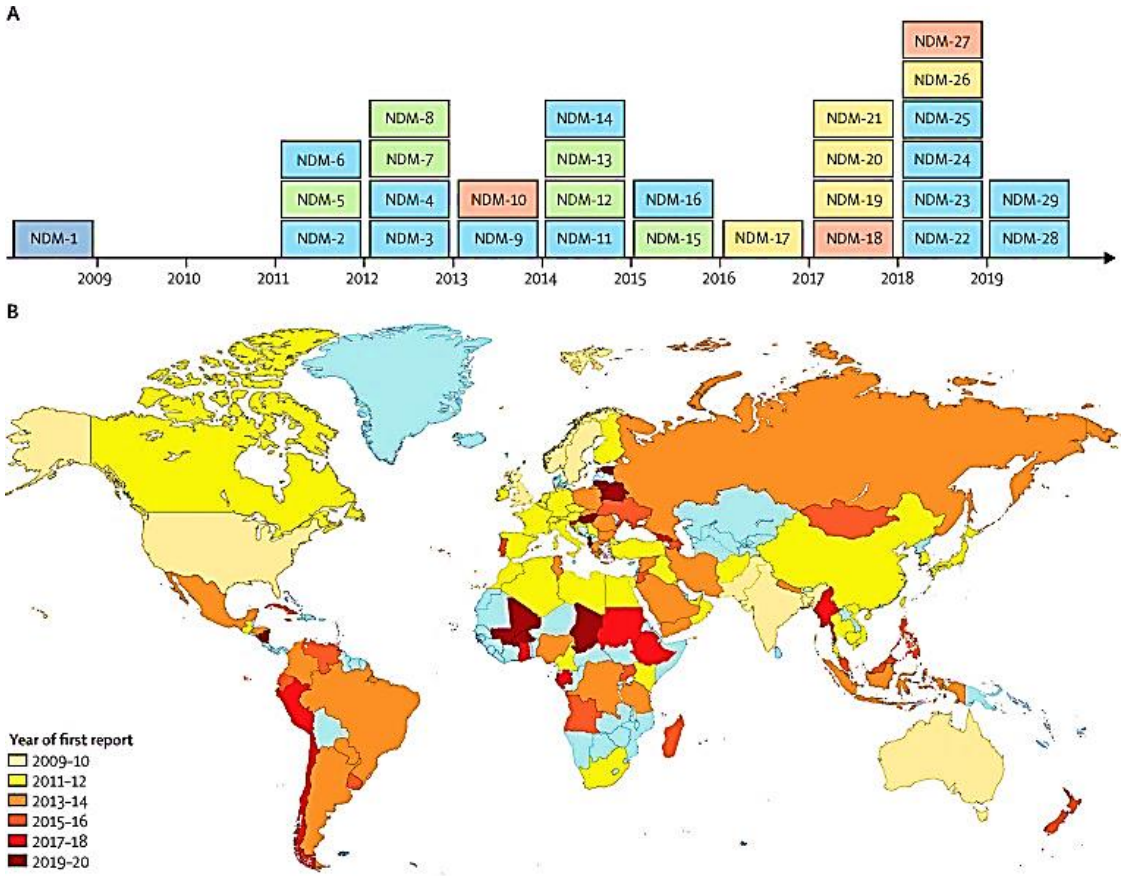

Fig. 4. Evolution and spread of NDM (Mojica, 2022): A – timeline of new NDM variants emergence. Number of amino acid substitutions compared to NDM-1: one (blue), two (green), three (yellow) and five (red). B – global dissemination of NDM beta-lactamases. The year of the first message is marked with color; explanations of the colors are given in the legend to the figure. Grey sections indicate areas with no reports. NDM = New Delhi metallo-beta-lactamase.

The work by Berglund and colleagues confirmed a clear separation between subclasses B_1 and B_2, but only less than 1% of the genes were classified as B_2. Subclass B_1 was further divided into five monophyletic groups, $B_{1.1}$–$B_{1.5}$, which largely reflected the taxonomy of metallo-beta-lactamase-hosting bacterial species ($B_{1.1}$ and $B_{1.2}$ belong to Proteobacteria (97%), $B_{1.3}$ belong to Bacteroidetes (98%), $B_{1.4}$ belong to Bacteroidetes (74%) and Firmicutes (24%), and $B_{1.5}$ belong equally to three groups: Firmicutes, Proteobacteria and Spirochaetes). Groups $B_{1.1}$ and $B_{1.2}$ have the highly mobile members located in VIM and IMP, whereas groups $B_{1.3}$ and $B_{1.4}$ do not contain highly mobile genes with rare exceptions (MYO-1 and CAM-1.28). Cluster $B_{1.5}$ included genes integrated in the plasmid-mediated SPM-1 structure (Berglund, 2021).

Subclass B_3 contains enzymes with atypical zinc binding sites and is divided into four monophyletic groups, $B_{3.1}$–$B_{3.4}$. Unlike B_1, the groups for B_3 do not correlate as well with taxonomy. The fourth group, $B_{3.4}$, is large but could be further divided into six clades (a–f) and all identified species were from the phylum Proteobacteria except for $B_{3.4e}$, where the species belong exclusively to Gemmatimonadetes. Cluster $B_{3.4}$ contained three new plasmid-borne gene families (Berglund, 2021).

Discussion

Antibiotic resistance is becoming an increasingly important public health problem, so both the general properties of this function and the individual mechanisms of its development are within the field of view of modern biologists. One of the strategies for bacterial resistance to antibiotics is the destruction of their molecules by hydrolytic enzymes, in particular the beta-lactam ring by beta-lactamases. Metallo-beta-lactamases differ significantly from beta-lactamases of other classes in the structure of the reaction center and the mechanism of their catalysis, which is based on the activity of zinc ions. The unique structure and high speed of distribution attract the interest of researchers to this group of bacterial enzymes.

ISSN 2075-5457 (print), ISSN 2220-9697 (online)

Today, the fact of the appearance of metallo-beta-lactamases twice has been practically proven, and the number of subclasses of these enzymes has been expanded to ten (due to the division of subclasses 1 and 3 into new groups). The high rate of horizontal transfer of these genes is associated with their incorporation into insertional sequences and integrons, and entry into conjugation plasmids.

Predicting the rate and direction of evolution of these enzymes requires the development of new tools that link molecular biology, biochemistry, epidemiology, computer technology, and probably artificial intelligence, and is of not only scientific interest, but also of great practical value for medicine.

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Еволюція метало-бета-лактамаз у фокусі проблеми антибіотикорезистентності

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У статті розглянуто місце метало-бета-лактамаз серед механізмів антибіотикорезистентності. Ці ферменти мають важливі відмінності від серинових бета-лактамаз, які впливають на механізм їх каталітичної активності, інгібування, швидкість поширення та еволюції. Описано історію вивчення та суспільну значущість антибіотикорезистентності в цілому та бета-лактамаз зокрема. Наведено класифікації ферментів, що гідролізують бета-лактамне кільце, описано структуру реакційного центру метало-бета-лактамаз та гіпотетичну модель його функціонування. Також розглянуто два принципово відмінних механізми інгібування метало-бета-лактамаз (цинк-залежний та цинк-незалежний) та їх наслідки для розробки терапевтичних стратегій. Метало-бета-лактамази поширювались серед непатогенних природних популяцій бактерій, а потім почали поширюватися на патогенних (спочатку грам-негативні), що обумовлює важливість їх вивчення з точки хору суспільного здоров'я. Висока швидкість поширення цих ферментів обумовлена їх локалізацією в структурах типу інтегронів, інсерційних послідовностей та кон'югаційних плазмід та може бути проілюстрована на прикладі ферменту NDM, вперше виділеному в Н'ю-Делі в 2009 р., який за десять років поширився по всьому світу та сформував три десятки мутантних варіантів. Майже доведеним є факт, що метало-бета-лактамази виникали в еволюції двічі. На сьогодні більш точно з'ясовані філогенетичні зв'язки між різними представниками цієї родини ферментів та встановлено наявність десяти монофілетичних груп. Наведені у статті дані можуть слугувати вихідною точкою для планування комплексних робіт з прогнозування еволюції метало-бета-лактамаз, які несуть серйозні ризики для лікування інфекційних захворювань.

Ключові слова**:** *механізми антибіотикорезистентності, молекулярна еволюція бактерій, молекулярні адаптації*

Цитування*: Moskalov V.B., Kadenko I.V., Mukhin A.M.. Evolution of metallo-beta-lactamases in focus of the antibiotic resistance problem. Вісник Харківського національного університету імені В.Н.Каразіна. Серія «Біологія», 2025, 44, с. 23-31. https://doi.org/10.26565/2075-5457-2025-44-3*

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Authors contribution: All authors have contributed equally to this work. / **Внесок авторів:** Усі автори зробили рівнозначний внесок у підготовку цієї роботи.

Conflict of interest: The authors declare no conflict of interest. / **Конфлікт інтересів**: Автори заявляють про відсутність конфлікту інтересів

Подано до редакції: 10.03.2025 / Прорецензовано: 22.04.2025 / Прийнято до друку: 26.05.2025