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An overview of the production of AmpC and Metallo-β-Lactamase enzymes in *Escherichia coli*

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Abstract

Escherichia coli is a Gram-negative, rod-shaped bacterium, responsible for 90% of all community-acquired infections and 50% of hospital-acquired infections, with opportunistic infections found in intensive care unit (ICU) patients. The β-lactam antibiotics, which inhibit cell wall synthesis, are known for their high efficacy and broad-spectrum activity. They also have low toxicity and provide long-term effects, making them widely used drugs against Gram-negative bacteria. Bacteria develop resistance to β-lactams primarily through the expression of hydrolytic enzymes, called β-lactamases, which are divided into serine β-lactamases (Classes A, C, and D) and metallo-β-lactamases (Class B), based on their molecular mechanism. This study aimed to clarify the mechanism of action of β-lactams against Gram-negative bacilli and to emphasize the multidrug resistance of cephalosporins and carbapenems to *E. coli*.

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Introduction

The expansion of multidrug resistance in pathogenic bacteria has now become a therapeutic challenge in the clinical health industry. Given the wide range of available antibiotics, β-lactams are widely used drugs against Gram-negative bacterial infections, as they exhibit high efficacy, broad-spectrum activity, and low toxicity (1). Penicillin was the first β-lactam antibiotic discovered by Fleming. It acts by binding to penicillin-binding proteins (PBPs), which are transpeptidase enzymes located in the bacterial peptidoglycan layer. This interaction with PBPs is what allows β-lactam antibiotics to induce these enzymes, causing damage to the bacterial cell wall and ultimately resulting in cell death (2).

The most probable cause of bacterial resistance to β-lactam antibiotics is the production of β-lactamase enzymes, which hydrolyze the 3-carbon and 1-nitrogen β-lactam ring (3) in antibiotics (4). The accepted theory regarding the origin of β-lactamase enzymes is a genetic modification in PBPs (2). The high prevalence of hospital-acquired infections among patients in the intensive care unit (ICU) presents a significant clinical challenge. When patients are administered high doses of β-lactam antibiotics, it can lead to the development of resistance in pathogenic bacteria. These bacteria mutate, resulting in various forms of mutated β-lactamases, such as extended-spectrum β-lactamase, AmpC β-lactamase, and metallo-β-lactamase (MBL) (5,1), which are usually resistant to third-generation penicillin, cephalosporin, and carbapenems (4).

Escherichia coli, a rod-shaped, Gram-negative bacterium from the Enterobacteriaceae family, is a type of coliform bacterium, which is a significant contributor to hospital-acquired infections (6). Several modern diagnostic procedures, such as endoscopic examination, catheterization, and intubation for ICU patients result in hospital-acquired infections, such as urinary tract infection (UTI), diarrhea, bacteriemia, neonatal septicemia, and urosepsis. Certain microorganisms, such as *E. coli*, are part of the normal flora, but can become pathogenic when an individual's immune response is compromised, thereby heightening the risk of opportunistic infections in hospitalized patients (7).

Phylogenetic analyses categorize *E. coli* strains into four groups: A, B1, B2, and D. The commensal strains of *E. coli* are typically found in groups A and B1. On the other hand, pathogenic strains, which are responsible for extraintestinal infections, are classified under groups D and B2. It is important to note that the strains in groups D and B2 are more susceptible to developing antibiotic resistance (8). Resistance to β-lactam antibiotics, which produce AmpC β-lactamase and MBL *E. coli*, is not only observed in humans. This resistance has been also found in dairy animals, livestock, and street dogs in South Korea. The resistance is linked to genes that are primarily located on integrons, transposons, insertion sequences, and plasmids. Notably, the blaCTX-M-14 gene was identified in 23 isolates of *E. coli* (9), raising significant concerns in the medical industry (10). This review provides a summary of the operational mechanism and resistance of β-lactam classes against pathogenic Gram-negative bacilli. It also discusses advanced therapeutic options used to combat AmpC β-lactamase and MBL-producing *E. coli.* Functional and molecular classification of beta lactamases and examples of beta-lactamase enzymes are mentioned in Table 1 and 2 (2, 5, 11-22).

1. Development of resistance to β-lactams (AmpC-β-lactamases and MBLs)

Over the past 50 years, the enzyme β-lactamase has garnered significant attention due to its clinical importance and its role in treatment failure. The first plasmid-mediated β-lactamase of the ESBL type was discovered in *E. coli* TEM-1 in Europe. Concurrently, a mutated variant was introduced, originating from the wild-type TEM-1 and SHV-1, found in *Klebsiella pneumoniae* SHV-2 in Germany. This variant has since been widely distributed (2). During this period, ESBLs were primarily associated with hospital outbreaks in the ICU, predominantly due to a specific strain of *K. pneumoniae*. The emergence of ESBLs in *E. coli* led to a shift in epidemiological conditions, involving various *E. coli* clones and diverse genetic elements carrying the *blaESBL* genes (14). The prevalence of these new enzymes, which have the potential to degrade third-generation cephalosporins, is on the rise in both European and Asian countries (2).

A new chapter unfolded when the plasmid-carrying AmpC cephalosporinases were found to originate from the same chromosomally mediated AmpC enzyme gene. These genes have spread globally within the Enterobacteriaceae family, posing a significant challenge. They inactivate cephamycin and other extended-spectrum cephalosporins, exhibit resistance to clavulanic acid, and contribute to higher morbidity and mortality rates in patients, causing a major concern in healthcare (23). A study reported that the prevalence of AmpC β-lactamases producing *E. coli* in India varies from 3.3% to 37.5%, depending on the geographical distribution. In other parts of the country, the prevalence of AmpC producing *E. coli* is reported as 6.97% in North India and 47.8% in the Eastern region. In the Southern part of the country, the prevalence is 3.3% in Karnataka and 9.2% in Chennai. This highlights the significant regional variation in the prevalence of this enzyme (24).

In 1991, Japan reported the discovery of the first Ambler class B imipenemase (IMP)-type MBL. The VIM type, on the other hand, originated in European countries (25). A survey conducted at the Hospital Universitari de Bellvitge in Barcelona, Spain, from 1996 to 2001, reported the first major outbreak of MBL blaVIM-2-producing *P. aeruginosa*. This strain was found to be resistant to all β-lactams and fluoroquinolones, marking a significant event in the study of antibiotic resistance (24,25). Another study conducted at the Alfred Hospital from January to July 2004 reported an outbreak in Australia. There were 19 isolates, including 10 isolates of *S. marcescens*, four isolates of *K. pneumonia*, three isolates of *P. aeruginosa*, one isolate from *E. cloacae*, and one isolate from *E. coli* producing the *blaIMP-4* gene (25,26). In 2003, Miriagou et al. reported the first instance of a self-transferable encoded plasmid VIM-1 MBL produced by the *E. coli* V541 strain. This strain was isolated from a patient's urine sample at Tzanion General Hospital in Piraeus, Greece, in November 2001 (27,28).

Lincopan et al. (2005) reported the first blaIMP-1 MBL carrying class 1 integron-producing *K. pneumoniae* in a 75-year-old patient in Brazil (29). A case study conducted by Tewari et al. reported a high prevalence of AmpC (34%) in *E. coli* strains that were resistant to cefoxitin (59%) and cefotetan (69%). Additionally, they found a 27% prevalence of carbapenem resistance in *E. coli*, with 29% resistance to imipenem and 31% to meropenem. These findings were reported at a hospital in South Bangalore, India (30). The rapid spread of bacterial resistance, mediated by these β-lactamases, is expanding swiftly in many countries with limited resources and few treatment options. This situation significantly complicates healthcare conditions. The excessive use of antibiotics plays a crucial role in the development and spread of resistance to third-generation cephalosporins and carbapenems. This resistance is escalating globally (Including India), not only in E. *coli,* but also in other members of the Enterobacteriaceae family (30).

Table 1. Functional and molecular classification of beta-lactamases

Functional classification			Molecular classification (Ambler classification)		Enzyme characteristics
Richmond-Sykes class	Mitsuhashi-Inoue type	Bush-Jacoby-Medeiros group			
I (a, b, d)	Cephalosporinase	1	(a)-Serine-β-lactamase class	C	This includes penicillin and cephalosporins enzymes that are not induced by the β-lactamase inactivator, clavulanic acid (AmpC, DHA, MOX, and MIR-1).
NL	Penicillinase V	2a		A	This includes narrow-spectrum penicillin enzymes, which are induced by β-lactamases (LEN-1) that are directed towards the active site.
II and III	Penicillinase I	2b		A	This includes broad-spectrum enzymes, including penicillin and cephalosporins, which can be induced by the β-lactamase inactivator, clavulanic acid (Specifically, TEM-1, TEM-2, and SHV-1).
IV	Cefuroxime-hydrolyzing β-lactamase	2be		A	This includes extended-spectrum enzymes, including penicillin, cephalosporins, and monobactam. These enzymes are effectively induced by the active site β-lactamase inactivator, clavulanic acid (Specifically, TEM-3 to TEM-26 and SHV-2 to SHV-6).
NL		2br		A	This includes broad-spectrum β-lactamases, which are not induced by the β-lactamase inactivator, clavulanic acid (Specifically, TEM-30 to TEM-36).
II and V	Penicillinase IV	2c		A	This includes enzymes, such as penicillin and carbenicillin, which are induced by the β-lactamase inactivator, clavulanic acid (Specifically, PSE-1, PSE-3, and PSE-4).
V	Penicillinase II & III	2d		D	This includes oxacillin enzymes, which are not induced by the β-lactamase inactivator, clavulanic acid (Specifically, OXA-1 to OXA-11 and PSE-2).
I (c)	Cefuroxime-hydrolyzing β-lactamase	2e		A	This includes cephalosporins enzymes that are induced by the β-lactamase inactivator, clavulanic acid (*Proteus vulgaris*).
NL		2f		A	This includes enzymes of the serine type carbapenem, specifically NMC-A.
NL		3	(b)-Zinc β-lactamase class	B	This includes metallo-β-lactamase enzyme, not inhibited by clavulanic acid and sulbactam (IMP, VIM, SPM, and NDM).
NL		4	-	-	-

*Abbrevations: AmpC, Cephalosporinase; DHA, Dhahran Hospital in Saudi Arabia; MOX, Moxalactam; MIR, Miriam Hospital in Providence; LEN, A Klebsiella Pneumoniae Strain; TEM, Temoniera; SHV, Sulfhydryl; PSE, Pseudomonas-Specific Enzyme; OXA, Oxacillin; NMC, Not Metalloenzyme Carbapenemases; IMP, Imipenemase; Vim, Verona Integron-Encoded Metallo-B-Lactamases; SPM, Sao Paulo Metallo-B-Lactamase; NDM, New Delhi Metallo-B-Lactamase, NL, classifications have not been named yet.

Table 2. Molecular classification and examples of beta-lactamase enzymes

Molecular classification	Beta-lactamase class	Subtypes	Origin of β-lactamases
Serine-β-lactamases	Class A (ESBL)	Plasmid-mediated TEM-1	It was identified in *Escherichia coli* (Europe, 1980s), *Salmonella paratyphi,* and later other pathogens, such as *Neisseria gonorrhoeae* and *Haemophilus influenzae.*
		Plasmid-mediated SHV-1	It was identified in *Escherichia coli* (1985).
		Chromosomal-mediated SHV-1	It was identified in *Klebsiella pneumoniae (*1985).
		CTX-M type	It was identified in the Enterobacteriaceae family (Germany, 1980s).
	Class C (AmpC)	Chromosomal-mediated AmpC	It was identified in *Psychrobacter immobilis* (Antarctic psychrophile, 1990s) and later in all members of the Enterobacteriaceae family, except *Klebsiella pneumoniae* and *Proteus vulgaris.*
		Plasmid-mediated CMY-1	It was identified in *Klebsiella pneumoniae* (South Korea, 1989).
		MIR-1 type	*Enterobacter cloacae* (USA, 1988)
		CMY/LAT type	*Citrobacter freundii* (Greece, 1993)
		DHA type	*Morganella morganii* (Saudi Arabia, 1992)
		ACC-1 type	*Hafnia alvei* (Germany, 1997)
		CMY/MOX/FOX type	*Aeromonas* spp. (USA & Japan, 1989-1991)
	Class D (ESBL)	OXA type	It was identified in *Enterobacteriaceae* spp. and *Acinetobacter baumannii* (1985).
Zinc-β-lactamases	Class B (MBL)	Both plasmid and chromosomal mediated	
	B1	Bc II	*Bacillus cereus* (1966)
		CcrA	*Bacteroides fragilis* (1990)
		BlaB	*Elizabethkingia meningoseptica* (1998)
		IND-1	*Chryseobacterium* spp. (1999)
		IMP-1	*Pseudomonas aeruginosa* (1994), *Shigella flexneri,* and *Klebsiella pneumoniae*
		VIM-1	*Pseudomonas* spp. (1999)
	B2	ImiS	*Aeromonas* spp. (1996)
		SFH-1	*Serratia fonticola* (2003)
	B3	THIN-3	*Janthinobacterium lividum* (2001)
		GOB-1	*Chryseobacterium meningosepticum* (2000)
		FEZ-1	*Legionella gormanii* (2000)

*Abbrevations: CTX-M, Cefotaxime first isolated in Munich; CMY, Cephamycins; LAT, Named after a patient; ACC, Ambler class C; Bc II, Bacillus cereus type II; Ccr A, Cefoxitin and carbapenem resistance; IND, Chryseobacterium indologenes (Flavobacterium); Imis, Imipenemase from Aeromonas veronii bv. sobria; SFH, Serratia Fonticola Carbapenem Hydrolase; THIN, Janthinobacterium lividum; GOB, Chryseobacterium Meningosepticum; class B FEZ, endozenous zinc β-lactamase of Legionella (Fluoribacter) gormanii.

3. Interaction of β-lactams with Gram-negative bacteria

For nearly seven decades, β-lactam antibiotics have been the keystone of human health (31). The advent of antibiotics in the 1930's significantly transformed the fight against infectious bacterial diseases (3). In the history of clinical medicine, β-lactams have provided extensive benefits through a process of continuous innovation. The most evident manifestation of this is the evolution of the internal structure of β-lactam subclasses. This evolution influences the ability of bacteria to develop innovative resistance mechanisms against each successive generation of β-lactams (31). The β-lactam antibiotics share a common fundamental structure known as the β-lactam ring. This ring is an integral part of the chemical structure of various β-lactam antibiotic families. It is a heterocyclic ring, formed by the cyclic amide group, which consists of three carbon atoms and one nitrogen atom. The classification of β-lactams is based on the presence or absence of this central ring structure (32). The β-lactam family was initially characterized as including penicillin-sulfur-containing penams, cephalosporin-sulfur-containing cephems, and monocyclic β-lactams, such as carbapenems, oxapenems, carbacephems, and oxacephems. The β-lactams are among the most significant classes of antibiotics, alongside macrolides and fluoroquinolones (13).

Penicillin is characterized by a bicyclic core structure, which is a nucleus of 6-aminopenicillanic acid. This nucleus is formed by the condensation of L-cysteine and D-valine and consists of a β-lactam ring and a five-membered thiazolidine ring. The modification of penicillin takes place in the acyl side chain that is attached to the C6 carbon atom. The penicillin class includes natural penicillins, such as benzylpenicillin, penicillin G, phenoxymethylpenicillin, and penicillin V. It also includes extended-spectrum penicillins, such as ampicillin (Aminopenicillin) and ticarcillin (Carboxypenicillin) (3,30,31).

Cephalosporins are composed of a 7-aminocephalosporanic acid nucleus, which is coupled with 3,6-dihydro-2H-1,3-thiazine side chains (3). They are derived from the fermentation products of the fungus *Acremonium chrysogenum*. These antibiotics are categorized into five generations, each with different properties and uses. The first generation includes cephalothin, cefapirin, cefazolin, cefalexin, cefradine, and cefadroxil. The second generation comprises cefamandole, cefuroxime, cefonicid, and ceforanide. The third generation consists of cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefoperazone, cefixime, ceftibuten, and cefdinir. The fourth generation includes cefpirome and cefepime, and the fifth generation encompasses ceftobiprole and ceftaroline (32).

Carbapenems represent the broadest-spectrum antibiotics among β-lactam drugs. They are characterized by a carbon atom, which replaces the sulfur atom on the five-membered ring attached to the β-lactam ring. Additionally, they have a hydroxyethyl side chain at the 6th position, which enhances their stability against β-lactamases. Due to their compact structure and size, these antibiotics can easily penetrate the cell membrane of Gram-negative bacteria. The first carbapenem, thienamycin, was produced by the bacterium *Streptomyces cattleya.* Imipenem, a chemically modified version, features a hydroxymethyl side chain, which is a departure from the classical acylamino side chain in penicillins and cephalosporins. The carbapenems that are commonly used worldwide include doripenem, ertapenem, imipenem, meropenem, and biapenem. Tebipenem, another type of carbapenem, is only available in Japan (30-32).

Four factors are involved in the antimicrobial activity of β-lactam antibiotics: (i) the concentration of the antibiotic, (ii) antibiotic diffusion through the outer cell membrane, (iii) the ability to resist the attack by inactivating enzymes, and (iv) the affinity of the antibiotic for the target enzyme. The β-lactams exhibit bactericidal activities by inhibiting the synthesis of the bacterial cell wall (33,34). Peptidoglycans and murein are the basic constituents of bacterial cell membrane stability. The structure is composed of alternating residues of β-1,4-linked N-acetylglucosamine (GluNAc) and N-acetylmuramic acid (MurNAc). This forms a heteropolymer, which consists of glycan chains that are cross-linked by short peptides. In Gram-negative bacteria, the carboxyl-terminal of each MurNAc residue is substituted with a pentapeptide subunit (C-1 to C-6). This subunit contains alternating L- and D-amino acids, along with one dibasic amino acid, typically meso-diaminopimelic acid (m-DAP) (30,32).

Lipid II, a biosynthetic building block, is another compound that stabilizes the polymeric structure of the cell wall. It is synthesized within the cell's cytoplasm and is located on the inner surface of the cytoplasmic membrane. The enzymatic reaction involved in this process is dependent on penicillin-binding proteins (PBPs). These PBPs are composed of four structural domains. The first domain is an amphipathic polypeptide sequence that gathers these enzymes to the outer surface of the plasma membrane in conjunction with lipid II. The second domain is a glycosyltransferase, also known as the catalyst domain. In this domain, lipid II is transferred to a new glycan chain. Simultaneously, undecaprenyl diphosphate is released as the leaving group. This complex process plays a crucial role in bacterial cell wall synthesis and stability.

The primary function of the third domain is to separate the second and fourth domains, both of which exhibit transpeptidase activity. The fourth domain serves as the molecular target for β-lactams. The final step in the assembly process of the plasma membrane involves the transpeptidation of adjoining glycan strands. This process uses m-DAP (Amine terminus) as the acyl-acceptor of one glycan, while releasing the D-Ala terminus of the second glycan. This cross-linked transpeptidase domain is the primary target site of β-lactam antibiotics. The β-lactams employ a mechanism of functional mimicry by modifying the acylation of the active site domain of PBPs. This mimics the D-Ala-D-Ala terminus (The core structure of transpeptidase) with the intrinsic acylation reactivity of its β-lactam structure. This mechanism is known as the Tipper-Strominger hypothesis, proposed by Strominger (31). By inhibiting the transpeptidase reaction, the structural integrity of the bacterial plasma membrane is compromised, leading to cell death or cell lysis. This mechanism is a crucial aspect of the action of certain antibiotics.

4. Mechanism of AmpC and MBL β-lactamase resistance in *E. coli*

The β-lactamase interferes with the β-lactam action by hydrolyzing the amide bond of the four-membered β-lactam ring (35,36). The structure of class C β-lactamase is a key aspect of β-lactamase classification. The active-site residues associated with the hydrogen-bonding network include Ser64, Lys67, Gln120, and Tyr150. These residues play a crucial role in the function and interaction of the enzyme with β-lactam antibiotics (18). The Tyr150 residue activates the acylation of Ser64 by serving as a proton acceptor for the hydrogen atom of Ser64. This action, in turn, triggers a nucleophilic attack on the carbonyl carbon atom of the β-lactam ring. Consequently, after accepting a proton from Ser64 during the formation of the tetrahedral intermediate, the Tyr150 residue returns the proton to the β-lactam nitrogen molecule, leading to the release of the tetrahedral intermediate. Subsequently, the same Tyr150 residue stimulates water molecules to facilitate the deacylation of the acyl-enzyme, thereby concluding the hydrolysis. This process results in the splitting of the β-lactam ring and the release of β-lactamase (12,13,34).

In the absence of inducers, AmpC β-lactamases typically exist in an inhibited state, but their expression is strongly induced in the presence of inhibitors. Normally, without an inducer, MurNAc tri- and tetrapeptide peptidoglycan degradation products are transported into the cytoplasm through the permease AmpG. These products are then separated into dehydro-monosaccharide peptides by the β-N-acetyl-glucosamidase NagZ. Subsequently, these tri- and tetrapeptides are separated from saccharide residues through the action of the N-acetyl-dehydromuramyl-L-alanine amidase AmpD. The peptides formed through this pathway are incorporated into the peptidoglycan synthesis process to form UDP-MurNAc pentapeptides. These pentapeptides are then transported to the periplasm and incorporated into the peptidoglycan layer, completing the normal recycling step.

In the absence of an inducer, the UDP-MurNAc pentapeptide binds to the AmpC transcriptional regulator AmpR, which acts as a repressor and inhibits the expression of the *ampC* gene (35,36). In the presence of an inducer, such as a β-lactam, the degraded peptidoglycan product accumulates and increases the muropeptide levels, which are then released into the periplasmic space. These muropeptides enter the cytoplasm through an AmpG transporter, where they compete with UDP-MurNAc for the binding site on AmpR. Changes in the UDP-MurNAc signals lead to alterations in AmpR, which in turn activate the hyperproduction of AmpC. Consequently, mutations (Specifically point mutations) in AmpD and AmpR are responsible for the production of AmpC β-lactamases (Figure 1) (23,35,36).

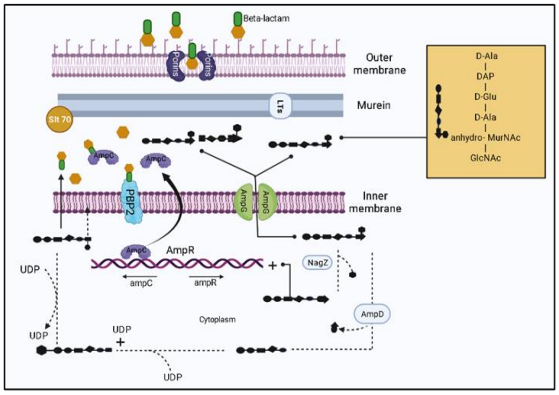

Figure 1. Mechanism of AmpC production in E. coli (14) (Using the BioRender)
*Abbreviations: Lts, Lytic transglucosylase; NagZ gene, GlcNAc amidase; Ala, Alanine; DAP, m-Diaminopimelic acid; Glu, Glutamate.

It is known that *E. coli* mediates resistance to broad-spectrum cephalosporins through the action of AmpC β-lactamases. This is achieved by generating a more dynamic promoter structure, leading to the high production of the endogenous *E. coli* AmpC gen. Additionally, *E. coli* can acquire plasmid-derived AmpC genes from other species, further enhancing its resistance capabilities (14). In clinically isolated *E. coli*, the AmpC cephalosporinase gene, which is located on the chromosome, cannot be induced due to the absence of the AmpR regulator gene. As a result, the expression of the AmpC gene in *E. coli* depends on the strength of the AmpC promoter. Typically, *E. coli* AmpC promoters contain two hexamers of conserved sequences in the -35 (TTGACA) and -10 (TATAAT) regions, also known as the Pribnow box, which plays a crucial role in gene transcription. Mutations at positions -10 and -35 in the AmpC promoter boxes, a mutation at -42 in the AmpC promoter linked with a mutation at the -18 sequence, and a mutation in the -11C to T transition can lead to the development of new alternative promoter boxes. These mutations can lead to high expression levels

of the chromosomal *E. coli* AmpC gene, which mediates resistance to cephalosporins (17,37,38).

Commonly, in *E. coli*, the AmpC promoter, which is typically strong, has undergone mutations at the -88, -82, -42, -18, -1, and +58 positions within its conserved sequences (39-41). AmpC genes, which are derived from plasmids, are found on plasmids ranging in size from 7 kb to 180 kb. While some of these plasmids are not capable of self-transmission, they can still transfer genes through processes, such as transformation and mobilization. In *E. coli*, resistance to cephalosporins is mediated by the low expression of the AmpC gene. This involves the mutation of AmpC variants, such as CMY, FOX, LAT, *E. coli* K12, DHA, and ACC. Presently, the CMY and DHA AmpC genes are frequently observed in *E. coli* (17,24).

Class B metallo-enzymes possess two binding sites for divalent Zn ions. The amide bond in the β-lactam ring is split through a nucleophilic attack on the carbonyl carbon atom of the β-lactam ring by the activated hydroxide Zn-1. This leads to the formation of an enzyme-substrate intermediate. The oxyanion, formed on the carbonyl oxygen of β-lactam, is stabilized by the Asn233 residue and Zn-1. Concurrently, Zn-2 interacts with the lone pair electron of the nitrogen atom of the β-lactam. The Asp120 residue accepts a proton from the activated water molecule within the enzyme and then transfers this proton to the nitrogen atom of an open β-lactam ring. This results in the release of the hydrolyzed substrate and the regeneration of the β-lactamase enzyme (12,35).

The MBLs of subclasses B1 and B3 have two binding sites for Zn ions (42). In the B1 subclass, the Zn-1 ion forms a tetrahedral coordination sphere that includes His116, His118, His196, and a water molecule. This water molecule also serves as a ligand for both Zn ions. The two binding sites are occupied by histidine and cystine. In the B1 enzyme, which includes BcII, VIM-2, and SPM-1, metal ions are found in the histidine binding site (43). In the B3 subclass, the Cys221 found at the Zn-2 site in the B1 subclass is replaced with a His ligand (42). Additionally, Zn-2 is ligated by Asp120, His121, His263, and nucleophilic water molecules, which form a bridge between the two Zn ions.

The GOB enzyme of the B3 subclass differs from other B3 enzymes due to point mutations that occur far from active sites, specifically Leu94Phe, Ala137Val, and Asp282Asn. Both B1 and B3 enzymes exhibit maximal activity when interacting with divalent Zn ion species. B2 enzymes, on the other hand, are non-competitively inhibited after binding to the Zn-2 ion, as histidine does not serve as the second metal-binding site. In the case of the B2 enzyme ImiS, the Zn-2 ion actively binds to the sulfur ligand formed from Met164 and His118. Cys221 is the only ligand of Zn-1, and a mutation of Met146 to Ile prevents inhibition by zinc. Other mutations in CphA residues do not interact with the Zn-2 ligand, as His118 and Met146 bind poorly to the same Zn ion (42-44). These conserved residues do not directly participate in catalysis. However, they play a crucial role in recognizing the substrate and maintaining the structure. Additionally, they actively engage in binding to Zn sites (45). The data suggests that the substitution of amino acid residues in CcrA, IMP-1, BcII, VIM-2, BlaB, and enzymes of the B3 subclass leads to the development of a mutation that confers resistance to carbapenems.

5. Clinical therapy and future approaches to curb the third and fourth generation β-lactam antibiotic resistance

Treatment of invasive bacterial infections is challenging, as most new drugs are not available in some countries and regions (46). Generally, strains that produce AmpC are resistant to multiple drugs, which complicates the selection of effective antimicrobial drugs. The combination of β-lactam and β-lactamase inhibitors, along with most cephalosporins and penicillin, should be avoided due to in vitro resistance. Carbapenems are the preferred drug for AmpC producers. A study conducted on a guinea pig model of pneumonia found that cefepime, imipenem, and meropenem are equally effective against porin-deficient FOX-5-producing *K. pneumoniae* strains. In a rat pneumonia model, β-lactams, such as imipenem, meropenem, ertapenem, or cefepime, yielded the same results against ACT-1 producing *K. pneumoniae* strains as they did in the guinea pig model. However, in a mouse model of pneumonia, cefepime showed superior results against *K. pneumoniae* strains (CMY-2) with porin deficiency. As a result, cefepime has been successful in treating infections caused by *Enterobacter* spp., including those with reduced susceptibility to ceftazidime (20).

In addition to the previously mentioned drugs, there are other classical and newer drugs that are active against AmpC producers in vitro. These include cephamycin and temocillin, which is a 6-α-methoxy derivative of ticarcillin. Other effective drugs include aminoglycosides, tigecycline, fosfomycin, and fluoroquinolones. Trimethoprim-sulfamethoxazole is also used in the treatment of these infections. These drugs provide additional options for combating resistant bacterial strains (23). Additionally, amdinocillin, when combined with clavulanic acid, has been found to be effective against *E. coli* strains that produce AmpC in vitro (20). Gutmann et al. reported a new derivative of penicillinate YTR 830 that shows a similar spectrum activity as clavulanic acid combined with amoxicillin against *Enterobacteriaceae* (47). The MBL inhibitors, such as polymyxins, colistin, tigecycline, and fosfomycin, are effective against carbapenem-resistant *E. coli* and carbapenem-resistant Enterobacterales infections in vitro. On the other hand, aminoglycosides, including gentamicin, amikacin, and tobramycin, exhibit a different spectrum of activity in vitro (47-49).

The Food and Drug Adminsitraton (FDA)-approved combination drug therapy, also known as fixed-dose combination therapy, is a novel approach aimed at curbing future bacterial resistance. Given the limited options for treating multi-drug resistant bacterial infections, combination therapy offers the best prospect for new drug discovery. The combination of antibiotics with non-antibiotic adjuvants and enhancers presents a promising and effective avenue for antibiotic discovery and development. In an era where the discovery and development of new antibiotics are at a historical low, revitalizing our existing drugs provides a fresh approach to prolonging the lifespan of our clinically approved drugs. Some FDA-approved combination therapies include Recarbrio (A combination of imipenem, cilastatin, and relebactam), Zerbaxa (Ceftolozane-tazobactam), and Avycaz (Ceftazidime-avibactam), all of which have proven effective in treating UTI. In situations where the infectious microorganisms are unknown and rapid treatment is urgently needed, the use of an appropriate dose of combined antibiotics is considered the best practice in the medical industry (50).

Conclusion

The accurate detection of AmpC and MBL production is critical in both hospital and community isolates. These strains are more prevalent than currently recognized strains and pose a significant threat to the efficacy of available beta-lactam drugs. The potential for outbreaks exists due to the selection pressure exerted by the extensive use of cephalosporins and carbapenem. Early identification of AmpC and MBL can aid in the establishment of a hospital-acquired infection control committee. This committee can guide physicians in prescribing the most suitable medications, such as FDA-approved combination drug therapy. This approach is one way to combat the growing resistance to antibacterial drugs. When the infectious microorganisms are unknown and immediate treatment is required, the use of an appropriate dose of combined antibiotics is a promising strategy in the medical industry.

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Author contributions

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