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B-LACTAM RESISTANCE IN ENTEROBACTERIACEAE: MOLECULAR MECHANISMS, EPIDEMIOLOGICAL PATTERNS, AND CLINICAL SIGNIFICANCE

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ABSTRACT

Members of the Enterobacteriaceae family, although natural constituents of the gut microbiota, are responsible for numerous severe infections, including bloodstream infections. β-lactam antibiotics have long represented the cornerstone of therapeutic management for these infections. However, extensive and often inappropriate use of these agents has led to the emergence and global spread of β-lactam-resistant Enterobacteriaceae. The principal mechanism of resistance involves the production of β-lactam–inactivating enzymes, notably extended-spectrum β-lactamases (ESBLs) and carbapenemases, which confer multidrug resistance and significantly restrict therapeutic options. Consequently, β-lactam resistance is associated with increased drug toxicity, higher mortality rates, and greater healthcare costs. This review summarizes current evidence on the molecular mechanisms and epidemiological trends of β-lactamase-mediated resistance among Enterobacteriaceae, emphasizing its clinical implications and public health impact.

Introduction

Since the clinical introduction of penicillin in the 1940s [1], β-lactam antibiotics have revolutionized the treatment of bacterial infections and remain among the most essential agents in modern antimicrobial therapy [2]. These drugs exert their bactericidal effect by disrupting bacterial cell wall biosynthesis. Specifically, they covalently bind to and inhibit transpeptidase enzymes, known as penicillin-binding proteins (PBPs), through acylation of a catalytic serine residue within their active sites—an essential process for the maintenance and growth of replicating bacteria [3]. Interestingly, most β-lactamases also possess an active site serine capable of being acylated by β-lactam molecules, highlighting significant structural and mechanistic parallels between PBPs and β-lactamases [4].

Currently, β-lactams constitute the most frequently prescribed class of antibiotics worldwide [5] and are divided into four major therapeutic groups: penicillins, cephalosporins, carbapenems, and monobactams. The first three share a bicyclic structure, in which the four-membered β-lactam (2-azetidinone) ring is fused to either a thiazolidine ring (penicillins), a six-membered dihydrothiazine ring (cephalosporins), or a five-membered pyrroline ring (carbapenems). Monobactams, by contrast, contain a monocyclic β-lactam structure. Extensive chemical modification of these natural scaffolds has yielded numerous semi-synthetic derivatives with improved potency, pharmacokinetic profiles, safety, and spectrum of activity [2]. Such developments have been critical for overcoming resistance mechanisms that emerged among target bacterial species [6].

In Gram-negative bacteria—particularly within the *Enterobacteriaceae* family, which includes many clinically significant pathogens—β-lactam resistance is predominantly mediated by β-lactamases that hydrolyze and inactivate the β-lactam ring [7]. Based on amino acid sequence homology, these enzymes are classified into four major groups: classes A, B, C, and D [8]. Classes A, C, and D β-lactamases belong to the serine hydrolase family, whereas class B enzymes, known as metallo-β-lactamases (MBLs), employ zinc ions for catalysis [9]. The hydrolytic process proceeds either through formation of a covalent acyl-enzyme intermediate between the β-lactam and the active site serine or via direct nucleophilic attack on the β-lactam carbonyl carbon by a zinc-activated hydroxide ion in MBLs [10]. Following hydrolysis and product release, the catalytic site is regenerated, enabling successive enzymatic turnovers [11]. Figure 1 illustrates representative β-lactamases from classes A–D, highlighting key catalytic residues and zinc coordination sites responsible for their hydrolytic mechanisms.

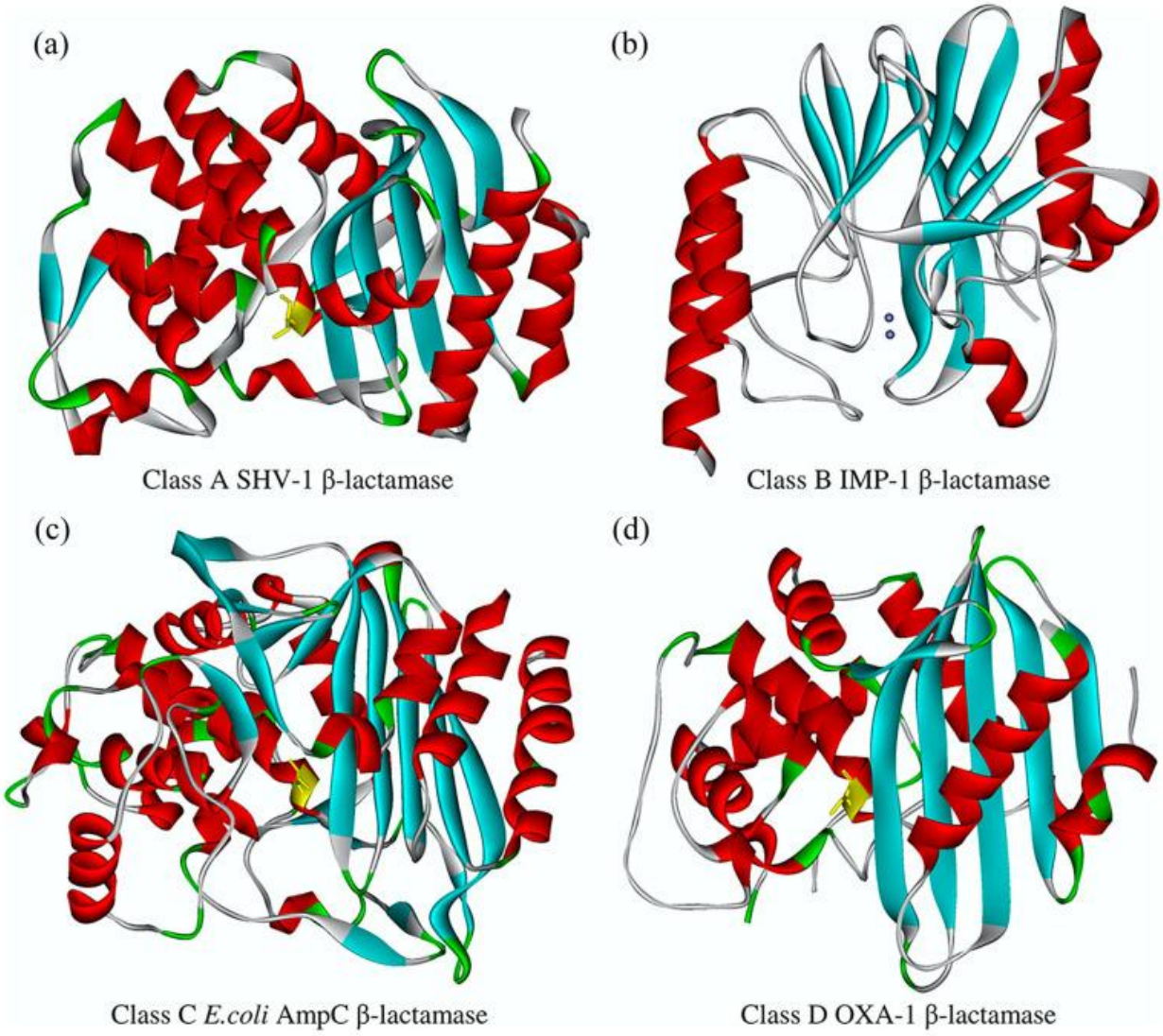

Figure 1. Overall structures of representative β-lactamases from the four major classes (adapted from reference 92, with permission).(a) Class A enzyme SHV-1 (sulfhydryl reagent variable-1); (b) Class B enzyme IMP-1 (imipenem-resistant Pseudomonas-1); (c) Class C enzyme AmpC (chromosomally encoded ampC β-lactamase); (d) Class D enzyme OXA-1 (oxacillinase-1). Active-site regions of serine β-lactamases are highlighted in yellow, whereas the zinc ions in metallo-β-lactamases are depicted as gray spheres.

The widespread clinical use of safe and effective β-lactam antibiotics has been accompanied by a dramatic increase in both the prevalence and diversity of β-lactamases over recent decades. The continuous evolution of these enzymes in clinical isolates has therefore become a major focus of recent comprehensive reviews [7,10]. To date, nearly 4,900 unique β-lactamases capable of hydrolyzing β-lactam antibiotics have been identified [12], and this number continues to rise—largely due to the accessibility of inexpensive, high-throughput gene sequencing technologies [10]. Among these enzymes, extended-spectrum β-lactamases (ESBLs) and carbapenemases are of greatest current clinical relevance [13], as infections caused by ESBL-producing (Enterobacteriaceae, ESBL-E) and carbapenemase-producing (Enterobacteriaceae, CPE) are strongly

associated with increased mortality, delayed initiation of effective therapy, prolonged hospital stays, and elevated healthcare costs [13,14].

With the conclusion of the 20th century "antibiotic golden age" [15], the global rise in antimicrobial resistance (AMR) represents a critical public health threat. It is projected that, by 2050, AMR-related infections could cause up to 10 million deaths annually [16]. In recognition of this escalating threat, the World Health Organization (WHO) published in 2017 a Global Priority Pathogens List, designating Enterobacteriaceae species as among the most urgent threats due to their extensive resistance patterns [17]. In particular, strains resistant to third-generation cephalosporins (e.g., ESBL-E) and carbapenems (CRE) were classified as critical-priority pathogens.

These organisms frequently harbor mobile multidrug resistance plasmids, enabling horizontal gene transfer both within and across bacterial species. Such genetic plasticity facilitates rapid dissemination of resistance determinants, complicates infection control strategies, and underscores the urgent need for novel therapeutic approaches [18,19].

The present review therefore aims to summarize the molecular mechanisms and epidemiological patterns underlying β-lactamase-mediated resistance to β-lactam antibiotics in Enterobacteriaceae, while also discussing the clinical implications and therapeutic challenges associated with this growing resistance.

β-Lactam Molecular Resistance in *Enterobacteriaceae*: β-Lactamases

Among Gram-negative bacteria, including Enterobacteriaceae, three major molecular mechanisms contribute to resistance against β-lactam antibiotics: (i) enzyme production, (ii) efflux pump overexpression, and (iii) porin channel modification [20]. Of these, enzymatic inactivation remains the most prevalent and clinically significant mechanism [21]. This process, occurring alone or in combination with the other mechanisms, is strongly associated with the multidrug-resistant (MDR) phenotypes frequently observed in clinical isolates [22].

To better categorize the diversity of β-lactamases, researchers have adopted a dual classification framework that integrates molecular and functional characteristics—respectively proposed by Ambler [8] and by Bush and Jacoby [23]. This combined approach allows for a more comprehensive understanding of the structural and catalytic diversity among β-lactamase enzymes.

As illustrated in Figure 2, β-lactamases—including AmpC (chromosomally encoded ampC β-lactamases), extended-spectrum β-lactamases (ESBLs), inhibitor-resistant β-lactamases, and carbapenemases—are divided into four major molecular classes (A, B, C, and D). Within these classes, the enzymes are further grouped into functional subcategories based on their substrate specificity and inhibitor profiles:

- Class C (subgroups 1 and 1e) — AmpC cephalosporinases;
- Class A (subgroups 2a, 2b, 2be, 2br, and 2f) — penicillinases, ESBLs, and inhibitor-resistant β-lactamases;
- Class D (subgroups 2de and 2df) — oxacillinases;
- Class B (subgroups 3a and 3b) — metallo-β-lactamases (MBLs).

Importantly, Class D β-lactamases (OXA-type enzymes) play a key role in conferring carbapenem resistance, particularly when acting synergistically with non-enzymatic

resistance mechanisms such as reduced outer membrane permeability or efflux pump activation. While these enzymes are increasingly detected among Enterobacteriaceae, they are especially prevalent in non-fermenting Gram-negative pathogens such as Acinetobacter baumannii and Pseudomonas aeruginosa [24].

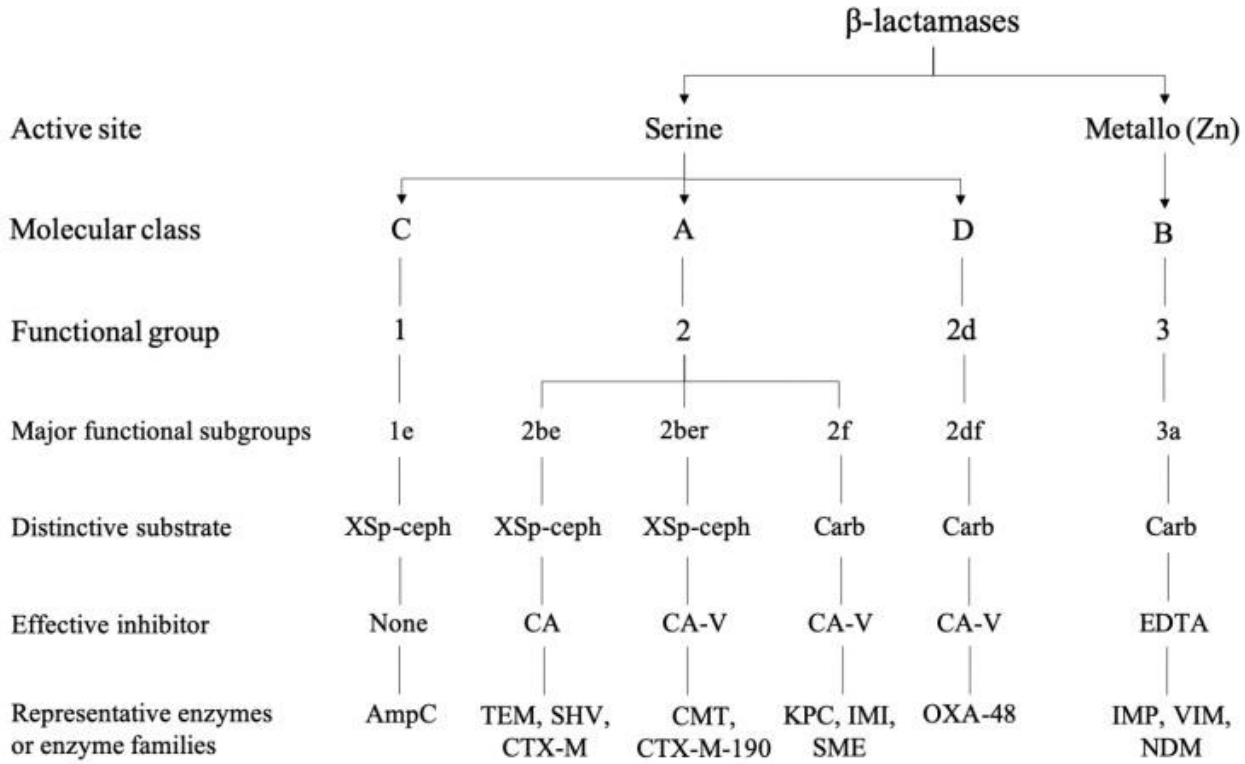

Figure 2. Molecular and functional relationships among β-lactamases conferring resistance to selected cephalosporins and carbapenems (adapted from reference 10, with permission).

Abbreviations: XSp-ceph, expanded-spectrum cephalosporins; Carb, carbapenems; CA, clavulanic acid; CA-V, variable response to clavulanic acid; EDTA, ethylenediaminetetraacetic acid.

Extended-spectrum β-lactamases (ESBLs) encompass several enzyme families, including TEM (Temoneira β-lactamase) and SHV (sulfhydryl reagent variable β-lactamase) types (subgroup 2be), as well as cephalosporinases with expanded hydrolytic spectra (subgroup 1e), the CTX-M (cefotaxime-hydrolyzing β-lactamase) family (subgroup 2be), and cephalosporin-hydrolyzing OXA enzymes (subgroup 2be). Similarly, carbapenemases comprise both serine-based enzymes (subgroups 2f and 2df) and metallo-β-lactamases (MBLs) (subgroups 3a and 3b).

As newer β-lactam antibiotics were introduced for clinical use, the hydrolytic spectrum of ESBLs expanded to include a broad range of β-lactam agents, such as penicillins, cephalosporins, and monobactams. Notably, many carbapenemases also hydrolyze expanded-spectrum cephalosporins; however, maintaining a clear distinction between ESBLs and carbapenemases remains clinically important due to differences in their catalytic mechanisms and inhibitor responses.

2.1. Extended-Spectrum β-Lactamases (ESBLs)

Within class A ESBLs, SHV- and TEM-type enzymes each comprise nearly 240 known variants, most of which are plasmid-encoded. These variants evolved from their parental forms (SHV-1, TEM-1, and TEM-2) through point mutations that alter specific amino acids in the enzyme's active site. This diversification emerged shortly after the introduction of β-lactam antibiotics containing an oxyimino side chain—such as aztreonam and third-generation (expanded-spectrum) cephalosporins—into clinical practice. Because oxyimino-cephalosporins are potent inhibitors of PBPs yet poor substrates for broad-spectrum β-lactamases, the emergence of TEM and SHV variants capable of hydrolyzing cefotaxime and ceftazidime represented a major adaptive shift in bacterial resistance.

A more recent and globally significant subgroup of class A ESBLs is represented by the CTX-M-type enzymes, first reported in the early 2000s. These enzymes have since become the most prevalent ESBLs among Enterobacteriaceae worldwide. They are currently divided into five major phylogenetic clusters: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. In contrast to TEM and SHV enzymes—both derived from plasmid-encoded penicillinases—CTX-M β-lactamases trace their origin to chromosomal β-lactamases of the genus Kluyvera. Mobile genetic elements, such as insertion sequences and occasionally bacteriophages, are thought to mediate the transfer of bla_{CTX-M} genes from Kluyvera chromosomes to conjugative plasmids, facilitating their rapid dissemination among pathogenic bacteria.

Other plasmid-encoded class A ESBLs identified in Enterobacteriaceae include SFO (Serratia fonticola β-lactamase), TLE (TEM-like enzyme), PER (Pseudomonas extended-resistant β-lactamase), BES (Brazil extended-spectrum β-lactamase), and GES (Guiana extended-spectrum β-lactamase) families.

Structurally, class A β-lactamases—including ESBLs and carbapenemases—share a conserved active site located between two β-sheet and α/β domains, which contains a distinctive Ω-loop structure. Within this loop, the residues Glu166 and Asn170 play critical roles in catalysis: Glu166 activates the catalytic water molecule required for deacylation, while Asn170 stabilizes its position. Other residues essential for catalysis and substrate binding—such as Ser70, Lys73, Ser130, and Lys234—are highly conserved across all class A enzymes.

Both acylation and deacylation steps depend on activation of the nucleophilic Ser70 residue and the hydrolytic water molecule, processes in which Glu166 acts as the general base. However, evidence suggests that Lys73 may also participate in serine activation during acylation, functioning in concert with Glu166. Structural and kinetic studies of TEM-1 interactions with penicillanic acid indicate that these residues operate cooperatively to enhance catalytic efficiency. Class A β-lactamases are often described as "near-perfect enzymes," a term reflecting their finely tuned ability to discriminate between substrates and products—ensuring rapid hydrolysis without self-inhibition by reaction intermediates. This kinetic precision underlies their exceptional catalytic proficiency and contributes to their clinical success as resistance determinants.

Current evidence indicates that TEM and SHV enzyme variants differ by only two or three amino acid substitutions within their coding regions, yet these subtle changes

significantly broaden their hydrolytic spectrum—from activity limited to penicillins and narrow-spectrum cephalosporins to potent activity against expanded-spectrum cephalosporins. As discussed in earlier reviews, the amino acid substitutions responsible for the ESBL phenotypes in TEM-1/TEM-2 and SHV-1 enzymes are located within critical structural regions of the active site, particularly the Ω-loop (amino acids 160–181) and the β3-strand (amino acids 229–240).

The two most characteristic substitutions, G238S and R164S, are considered core determinants of the TEM/SHV ESBL phenotype. These mutations frequently occur in combination with additional substitutions—such as E104K, M182T, A237T, or E240K—that further refine catalytic performance. Structural studies show that G238S and R164S induce conformational rearrangements in the Ω-loop and β3-strand, thereby expanding the active-site cavity to better accommodate oxyimino-cephalosporins such as cefotaxime or ceftazidime. Specifically, G238S promotes movement of the β3-strand or Ω-loop, while R164S generates a cavity within the Ω-loop, both enhancing substrate binding.

The M182T substitution—known as a compensatory or "suppressor" mutation that restores protein folding and stability—also increases catalytic efficiency toward oxyimino-cephalosporins. Despite its location distant from the active site, this residue likely counterbalances destabilizing effects introduced by ESBL-associated substitutions, thus improving overall enzyme performance.

In contrast to TEM and SHV enzymes, CTX-M-type β-lactamases share only about 40% amino acid identity with those families, though they exhibit over 70% identity within their own subgroup. Functionally, CTX-M enzymes display a markedly increased hydrolytic efficiency toward cefotaxime, indicating that their activity against expanded-spectrum cephalosporins is an intrinsic catalytic property rather than the result of isolated point mutations.

Key residues, notably Ser237 and Arg276, determine CTX-M specificity for cefotaxime hydrolysis. These residues are absent in most other class A β-lactamases and together shape the geometry of the active site around Asn170 and Asp240 to favor cefotaxime binding. Structural analyses reveal that cefotaxime interaction induces subtle conformational changes that disrupt the hydrogen bond connecting Asn170 and Asp240, effectively opening the catalytic pocket.

Although cefotaxime is readily hydrolyzed by CTX-M enzymes, ceftazidime—another oxyimino-cephalosporin—remains a poor substrate for most wild-type variants. However, amino acid substitutions such as D240G and P167S enhance ceftazidime hydrolysis by repositioning the aminothiazole ring within the active site to optimize contact. Additional substitutions located away from the catalytic region (e.g., A77V and N106S) can further boost activity when combined with P167S or D240G, likely by improving enzyme thermostability and expression efficiency—an effect reminiscent of the M182T mutation in TEM and SHV variants.

Class A β-lactamases, including most ESBLs, are generally susceptible to inhibition by clinically available β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam, and avibactam. Nonetheless, certain amino acid substitutions can confer resistance to inhibitors or increase hydrolytic activity, giving rise to inhibitor-resistant

variants. When such substitutions occur in TEM-1 or TEM-2 backgrounds that already harbor ESBL-defining mutations, the resulting enzymes are classified as Complex Mutant TEM (CMT) β-lactamases (e.g., TEM-50, TEM-151, TEM-152, TEM-158).

In addition to structural modification, enzyme overproduction contributes to enhanced resistance. Experimental evidence from Escherichia coli clinical isolates carrying bla_{TEM} suggests that exposure to β-lactam/inhibitor combinations such as piperacillin/tazobactam can increase gene copy number and transcriptional activity of bla_{TEM}, thereby amplifying resistance expression. Genome analyses have even identified bla_{TEM} duplications—ranging from several to dozens of copies—in such isolates, underscoring the dynamic genomic adaptability of these resistance determinants.

The emergence of CTX-M-190 marked the first report of an inhibitor-resistant CTX-M variant in clinical Escherichia coli isolates. Together with CTX-M-199, this enzyme retained hydrolytic activity against expanded-spectrum cephalosporins while demonstrating reduced susceptibility to β-lactamase inhibitors. Both variants originated from single amino acid substitutions—specifically, a Serine-to-Threonine replacement at position 130 in the parent enzymes CTX-M-55 and CTX-M-64, respectively. Structural and biochemical studies on CTX-M-199 provided insight into this resistance mechanism: mass spectrometry and crystallographic analyses revealed no major alterations in the enzyme's active site compared with CTX-M-64. However, the binding of sulbactam to the active site was insufficient to form a stable enzyme–inhibitor complex, thereby rendering inhibition ineffective.

2.2. Carbapenemases

Class A carbapenemases constitute one of the most clinically significant enzyme groups responsible for resistance to carbapenems. These include KPC (Klebsiella pneumoniae carbapenemase), SME (Serratia marcescens enzyme), Nmc-A (non-metallo-carbapenemase A), IMI (imipenemase), and GES (Guiana extended-spectrum β-lactamase) types. Among these, SME and Nmc-A are typically chromosomally encoded, KPC and GES are plasmid-borne, while IMI enzymes have been identified in both forms.

All class A carbapenemases share the capacity to hydrolyze carbapenems while avoiding inhibitory interactions with these antibiotics. Certain CTX-M enzymes have even evolved to acquire carbapenemase activity, such as the CTX-M-33 derivative of CTX-M-15, which harbors an Asn-to-Ser substitution at position 106.

Structurally, class A carbapenemases can be distinguished from other β-lactamases by the presence of a disulfide bridge between Cys69 and Cys238. Although this disulfide bond contributes to the structural stability of the enzyme, it is not absolutely essential for catalytic activity. Disruption of the bond through mutation destabilizes certain enzymes such as Nmc-A, while others—like GES-5 with a C69G substitution—remain catalytically functional despite reduced stability.

The Cys69–Cys238 disulfide bridge likely assists in orienting carbapenem molecules correctly within the active site to enable efficient hydrolysis. The spatial requirements for carbapenem binding are more stringent than for other β-lactam substrates, and expansion of the active-site cavity may inversely correlate with carbapenemase activity.

For instance, KPC variants with enhanced activity against bulky oxyimino-cephalosporins, such as ceftazidime, often exhibit reduced efficiency in hydrolyzing carbapenems. Similarly, GES-type enzymes lacking Ser170 cannot hydrolyze carbapenems, as this residue is crucial for maintaining hydrogen bonding with Glu166, ensuring proper alignment for the deacylation step.

Class B β-lactamases, also known as metallo-β-lactamases (MBLs), are zinc-dependent enzymes belonging to a large metallohydrolase superfamily that acts on a variety of biological substrates. In MBLs, the metal center is located at the junction of two β-sheets forming the enzyme's core. Based on their active-site architecture and metal coordination, MBLs are divided into three subclasses: B1, B2, and B3.

Enzymes belonging to subclasses B1 and B3 possess binuclear zinc centers composed of two metal-binding sites: Zn1 (tri-His motif) and Zn2 (Cys–His–Asp in B1, or His–His–Asp in B3). These sites are flanked by flexible loops—namely the L3 loop (hydrophobic) and L10 loop (hydrophilic)—that participate in substrate binding and catalytic turnover. Two water molecules complete the zinc coordination: one "bridging" water connecting both zinc ions and another "atypical" water coordinated to Zn2.

Functionally, MBLs exhibit an exceptionally broad substrate spectrum, hydrolyzing penicillins, cephalosporins, and carbapenems—though monobactams remain stable to their action. Unlike class A carbapenemases, MBLs are not inhibited by conventional β-lactamase inhibitors.

Clinically, the most important MBLs are those acquired via mobile genetic elements, particularly members of subclass B1, which include NDM (New Delhi metallo-β-lactamase), VIM (Verona imipenemase), and IMP (imipenem-resistant Pseudomonas enzyme). While many variants of these enzymes have been reported worldwide, only a subset occur in Enterobacteriaceae, though their spread poses a major threat due to their high transmissibility and broad resistance profile.

Despite extensive investigation, the precise catalytic mechanism of MBLs remains incompletely elucidated. Structural variability, even among enzymes of the same subclass, complicates the identification of a unified mechanistic model. Studies of NDM-1 crystal structures complexed with hydrolyzed carbapenems (such as imipenem and meropenem) suggest that hydrolysis proceeds through a unique pathway involving protonation of the reaction intermediate by a water molecule entering from the β-face of the substrate. Mutational analyses have identified specific amino acid positions that disproportionately affect carbapenem hydrolysis relative to other β-lactam substrates, underscoring the fine structural tuning of the active site.

Investigations of the L3 loop, a mobile flap that alters conformation upon substrate or inhibitor binding, have revealed its role in regulating catalytic turnover. Substituting this loop in the NDM-1 framework alters the rate of protonation of anionic intermediates, influencing hydrolysis efficiency without significantly changing substrate specificity.

Distinct structural features also differentiate other MBL families. For instance, VIM-type enzymes exhibit variation at residues 224 and 228, which appear to influence substrate positioning. Crystal structures of VIM-1 complexes with both inhibitors and hydrolyzed carbapenems demonstrate a water-mediated hydrogen bond linking the

substrate's carboxylate group to the carbonyl backbone of Cys221, one of the Zn2-coordinating residues. This suggests that Cys221 may perform a catalytic role analogous to that of conserved Lys224 in other MBL families.

Originally described as enzymes active primarily against penicillins, class D β-lactamases were designated "oxacillinases (OXA)" because they hydrolyze oxacillin at least half as efficiently as benzylpenicillin. Over time, the OXA family has expanded dramatically and now includes more than 250 known enzymes with activity against penicillins, cephalosporins, expanded-spectrum cephalosporins (OXA-type ESBLs), and carbapenems (OXA-type carbapenemases). Members of this class exhibit considerable variability in their susceptibility to β-lactamase inhibitors.

Among the five major OXA carbapenemase groups, OXA-23, OXA-24/40, OXA-51, and OXA-58 are predominantly associated with resistance in Acinetobacter baumannii, whereas OXA-48-type enzymes occur primarily on plasmids within Enterobacteriaceae. Comparative sequence analysis indicates that OXA-48 enzymes share more than 90% amino acid identity with chromosomally encoded oxacillinases from aquatic Shewanella species, suggesting that these environmental bacteria were the evolutionary origin of OXA-48-like enzymes.

Catalytically, OXA β-lactamases employ carboxylation of a conserved lysine residue (equivalent to Lys73 in class A enzymes) as a critical step in catalysis. The carboxylated lysine acts as a general base, activating the nucleophilic serine required for β-lactam hydrolysis—functionally analogous to the role of Glu166 in class A enzymes—and participates in both acylation and deacylation steps of the reaction.

Structural adaptations in the active site have enabled carbapenem-hydrolyzing class D β-lactamases (CHDLs) to develop activity against imipenem. Studies of OXA-58, a prominent CHDL from A. baumannii, revealed that reduced steric hindrance along the deacylating water pathway allows tighter substrate binding and enhanced turnover of carbapenems. Structural investigations have further highlighted a hydrophobic bridge spanning the active site as an important determinant of carbapenemase activity.

Interestingly, OXA-48-type enzymes, which are phylogenetically distinct from A. baumannii CHDLs, lack this hydrophobic bridge, consistent with their retained activity against oxacillin—a substrate poorly hydrolyzed by other OXA carbapenemases. Additional research demonstrated that CHDLs can expel water molecules from the active site upon acylation, necessitating recruitment of a new deacylating water molecule from the surrounding medium. Consequently, carbapenem turnover may depend on water accessibility to the active site, possibly facilitated by conformational changes in the acyl-enzyme complex. This access occurs through a channel formed by displacement of conserved hydrophobic residues. Notably, a pre-existing but narrower version of this channel has been identified in OXA-48, where minor shifts in nearby residues slightly widen the pathway to permit water entry.

2.3. Extended-Spectrum AmpC Cephalosporinases

Class C (AmpC) β-lactamases are typically encoded by chromosomal ampC genes—such as CMY-2, P99, ACT-1, and DHA-1—in Enterobacter and Citrobacter species.

However, plasmid-mediated AmpC enzymes are increasingly prevalent among Klebsiella and Salmonella species within the Enterobacteriaceae family.

Expression of AmpC β-lactamases may be either repressed (low-level) or inducible, for example following exposure to cefoxitin, which leads to derepression. This expression confers resistance to aminopenicillins and early-generation cephalosporins such as cephalothin, cefuroxime, and cefoxitin. Spontaneous mutations in regulatory genes can result in constitutive overexpression of AmpC, producing resistance to expanded-spectrum cephalosporins, including oxyimino-cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime. AmpC enzymes exhibit weak activity against cefepime and are inhibited by cloxacillin, oxacillin, and aztreonam.

In Escherichia coli, the chromosomal ampC gene is usually silent because of a weak promoter and the presence of a transcriptional attenuator. However, when plasmid-mediated ampC genes (e.g., bla_{CMY}, bla_{FOX}, bla_{DHA}, bla_{ACC}, bla_{ACT}, bla_{MIR}, bla_{MOX}) are introduced, expression becomes constitutive, conferring stable resistance to β-lactam antibiotics.

Although structurally related to narrow-spectrum cephalosporinases, extended-spectrum AmpC (ESAC) enzymes differ through specific amino acid insertions, deletions, or substitutions, enabling them to hydrolyze penicillins, cephamycins, and third-generation cephalosporins, while remaining inactive against carbapenems. Unlike ESBLs, ESAC enzymes are not inhibited by traditional β-lactamase inhibitors.

Since the first report of plasmid-mediated AmpC β-lactamases in 1989, these enzymes have been classified into several phylogenetic families, including CMY (cephamycin-hydrolyzing β-lactamase), MIR (Miriam Hospital β-lactamase), MOX (moxalactam-hydrolyzing β-lactamase), LAT (latamoxef-hydrolyzing β-lactamase), FOX (cefoxitin-hydrolyzing β-lactamase), DHA (Dhahran Hospital β-lactamase), ACT (AmpC-type β-lactamase), ACC (Ambler class C β-lactamase), and CFE (Citrobacter freundii β-lactamase). These families differ by subtle amino acid variations, particularly among Klebsiella pneumoniae isolates.

Mutations in both chromosomal AmpC enzymes (e.g., GC1 from Enterobacter cloacae) and plasmid-encoded AmpC variants (e.g., CMY-10) have been shown to enhance catalytic efficiency toward oxyimino-β-lactams, thereby expanding their resistance spectrum (subgroup 1e)

The molecular architecture of AmpC β-lactamases comprises two main domains: a small helical domain containing three α-helices with connecting loops, and a mixed α/β domain forming the core of the enzyme. The active site is divided into two key regions: the R1 region, which accommodates the C7 side chain of β-lactam substrates and is surrounded by the Ω-loop; and the R2 region, bordered by the R2 loop and helices H-10 and H-11.

Mutations occurring in structural regions adjacent to the Ω-loop—particularly within helices H-10 and H-11—are closely linked to the development of extended-spectrum AmpC (ESAC) β-lactamase activity. For instance, the evolution of the CMY-2 enzyme (the most prevalent plasmid-mediated AmpC β-lactamase) into its extended-

spectrum variant CMY-33 has been documented in clonally identical Escherichia coli isolates recovered from a patient previously treated with cefepime. The CMY-33 variant differs from CMY-2 by a two–amino acid deletion (Leu293–Ala294) within the H-10 helix. This alteration modifies both the size and flexibility of the active site, likely by increasing the distance between the catalytic Ser64 and the H-10 helix, thereby enhancing the enzyme's ability to hydrolyze expanded-spectrum cephalosporins.

Experimental evolution studies have confirmed these adaptive dynamics under antibiotic pressure. When an E. coli strain carrying a CMY-2-encoding plasmid was cultured in subinhibitory concentrations of cefepime, sequential mutations emerged within the H-10 helix. After multiple passages, a new variant, CMY-69, appeared, characterized by an Ala294Pro substitution. This mutation coincided with a progressive rise in cefepime resistance, with the minimum inhibitory concentration (MIC) exceeding 256 mg/L after approximately 30 passages. Parallel molecular analyses revealed increased ampC transcription levels and plasmid copy number, correlating directly with the enhanced MIC values observed.

These findings highlight how structural plasticity within the H-10 helix region and gene amplification mechanisms synergistically contribute to the evolution of high-level cephalosporin resistance in plasmid-mediated AmpC β-lactamases.

3. β-Lactamase-Mediated Resistance in *Enterobacteriaceae*: Epidemiology

Historical Overview

Table 1 summarizes the emergence of major β-lactamase families and their associated bacterial hosts across different countries between 1963 and 2006, highlighting the global evolution of β-lactam resistance.

Table 1 Overview of most important β-lactamases and relative microorganisms at their first appearance (adapted from reference 10 with permission).

Original (Current) Name	Microorganism	Year (Country) of First Isolation
TEM-1	*Escherichia coli*	1963 (Greece)
SHV-1	*Klebsiella pneumoniae*	1972 (Unknown)
Transferable ESBL (SHV-2)	*Klebsiella pneumoniae*	1983 (Germany)
Serine (class A, group 2f)	*Serratia marcescens*	1982 (England)
Carbapenemase (SME-1)	*Serratia marcescens*	1985 (United States)
FEC-1 (CTX-M-1)	*Escherichia coli*	1986 (Japan)
Plasmid-encoded AmpC (MIR-1)	*Klebsiella pneumoniae*	1988 (United States)
Plasmid-encoded MBL (IMP-1)	*Serratia marcescens*	1988 (Japan)
Inhibitor-resistant TEM (TEM-30)	*Escherichia coli*	1991 (France)

KPC-type (KPC-2)	*Klebsiella pneumoniae*	1996 (United States)
NDM-1	*Klebsiella pneumoniae*	2006 (India)

Since the early 1990s, Klebsiella pneumoniae isolates producing TEM and SHV extended-spectrum β-lactamases (ESBLs) have become the predominant agents of nosocomial outbreaks worldwide. Among these, enzymes such as TEM-10, TEM-12, TEM-26 and SHV-2, SHV-5, SHV-7, and SHV-12 were the most frequently detected in clinical environments, marking the first wave of hospital-associated ESBL dissemination.

By the early 2000s, a second wave of resistance emerged with the global spread of CTX-M-type β-lactamases, initially identified in Escherichia coli isolates from community-acquired infections. These enzymes rapidly replaced TEM and SHV types as the dominant ESBLs within Enterobacteriaceae. Among them, CTX-M-15 has achieved worldwide prevalence and now represents the most common ESBL in Europe, Asia, Africa, and North America. In East and Southeast Asia, CTX-M-14 is particularly widespread in E. coli isolates, especially in Japan and South Korea, while both CTX-M-14 and CTX-M-15 are prevalent in China. In contrast, CTX-M-2 predominates in South America, reflecting regional evolutionary and epidemiological divergence of ESBL genes.

Toward the late 1990s and early 2000s, the discovery of KPC (Klebsiella pneumoniae carbapenemase) enzymes signaled the onset of a new global challenge: carbapenemase-producing Enterobacteriaceae (CPE). The enzymes KPC-2 and KPC-3 have since become the most prevalent variants, predominantly found in K. pneumoniae but also in E. coli, Citrobacter, and Enterobacter species. High endemic rates of KPC-producing strains have been documented in Southern and Eastern Europe (notably Italy and Greece), South America (Brazil and Colombia), and parts of East Asia, particularly China.

In parallel, other carbapenemase families such as NDM (New Delhi metallo-β-lactamase), GES, VIM (Verona imipenemase), and IMP (imipenem-resistant Pseudomonas) have disseminated globally. The NDM family gained international attention following its first identification in a Swedish patient who had previously received medical treatment in India. Subsequent surveillance has revealed NDM-producing Enterobacteriaceae in patients with travel or hospitalization histories in South Asia, as well as in Europe, China, and the Middle East.

VIM-type enzymes have also achieved widespread distribution, though they typically appear in sporadic hospital outbreaks within Europe. VIM-2 remains the most frequently encountered variant worldwide, while VIM-1-producing K. pneumoniae and E. coli are especially common in Greece, which has been recognized as a significant regional reservoir for VIM-type carbapenemases. Over the past decade, Hungary, Italy, and Spain have experienced interregional spread of VIM-producing strains, whereas countries such as Saudi Arabia, Mexico, Taiwan, and the United States have reported only sporadic occurrences.

The IMP family of enzymes, initially identified in Serratia marcescens from isolates in the South Pacific and Asia, has since been detected in K. pneumoniae, E. coli, and Enterobacter species worldwide. While IMP-type β-lactamases have been reported globally, they remain endemic primarily in Japan and Taiwan, where they continue to serve as major contributors to carbapenem resistance in clinical isolates.

First detected in Turkey in 2001, the OXA-48-type β-lactamases constitute the third major globally disseminated group of carbapenemases, following KPC and NDM families. This group includes the canonical OXA-48 enzyme and its variants OXA-181 and OXA-232.

Outbreaks caused by OXA-48-producing Enterobacteriaceae have primarily occurred across Central and Southern Europe, with notable reports from France and Spain. The OXA-181 variant, on the other hand, has spread extensively throughout the Indian subcontinent, South Africa, and Singapore, as well as among patients with recent epidemiological links to these regions. Notably, OXA-181 is frequently co-produced with NDM-type carbapenemases, a phenomenon particularly common in India, reflecting overlapping reservoirs and transmission routes.

While non-Enterobacteriaceae organisms such as Stenotrophomonas maltophilia and Proteus species naturally lack ampC genes, several key Enterobacteriaceae members—including Klebsiella pneumoniae, Klebsiella oxytoca, and Salmonella spp.—are also characterized by the absence of chromosomal bla_{AmpC} genes. In E. coli, resistance mediated by plasmid-encoded CMY-2 enzymes is typically accompanied by susceptibility to cefepime. However, cefepime-resistant E. coli strains producing extended-spectrum AmpC (ESAC) β-lactamases can arise through two to four amino acid deletions within the H-10 helix of CMY-2, resulting in a structural modification that broadens substrate specificity.

Moreover, co-expression of plasmid-mediated AmpC enzymes and ESBLs in a single isolate presents a major diagnostic and therapeutic challenge. The high-level production of AmpC can mask the presence of ESBLs, while the coexistence of both resistance mechanisms often results in extreme β-lactam resistance, limiting treatment options even further.

3.2. Molecular Overview of β-Lactamase Dissemination

The horizontal transfer of β-lactamase genes is largely facilitated by mobile genetic elements (MGEs) such as transposons, insertion sequences, and integrons. These elements play a pivotal role in the rapid spread of resistance genes among Enterobacteriaceae and between environmental, animal, and human reservoirs. Bacteria present in water, soil, and sewage environments serve as major genetic exchange hubs, promoting the transmission of ESBL genes across ecological boundaries.

Environmental surveillance studies have detected ESBL-producing organisms in urban wastewater, sewage systems, hospital sink drains, livestock farms, and even retail meat products, demonstrating the extent of environmental contamination. Metagenomic analyses suggest that MGE-mediated transfer of ESBL genes between environmental bacteria, animals, and humans occurs most frequently in developing regions, where antibiotic misuse and inadequate sanitation exacerbate the problem.

International travel also contributes significantly to the spread of resistant bacteria. The highest rates of ESBL acquisition are observed among travelers to South and Southeast Asia, regions regarded as the primary epicenters of CTX-M-type ESBL gene evolution and dissemination. Similarly, community-acquired infections caused by NDM-producing Enterobacteriaceae may result from cross-contamination during food handling, exposure to bodily fluids, or contact with infected animals.

In contrast, the spread of OXA-48-producing Enterobacteriaceae is predominantly healthcare-associated, arising through nosocomial transmission. However, the emergence of strains co-producing OXA-48-like and NDM enzymes poses a particularly serious threat, as these combinations facilitate potential community-level transmission, especially in regions with limited infection control and hygiene infrastructure.

Epidemiological studies reveal that outbreaks of β-lactamase-producing bacteria may stem from either a single successful clone or the horizontal spread of resistance plasmids among unrelated strains. The pattern of dissemination often differs between enzyme families. TEM and SHV genes tend to propagate via clonal expansion, producing distinct epidemic outbreaks, whereas CTX-M-type genes exhibit an “allodemic” pattern, characterized by simultaneous spread across multiple genetic backgrounds.

Molecular typing, particularly multilocus sequence typing (MLST), has identified several major sequence types (STs) associated with ESBL production. In Escherichia coli, five predominant STs have been reported: ST131, ST405, ST38, ST10, and ST648. In Klebsiella pneumoniae, the leading STs are ST11, ST14, and ST15. Among these, E. coli ST131 stands out as a high-risk international clone—an extraintestinal pathogenic lineage strongly associated with CTX-M-15 production and global dissemination.

Whole-genome sequencing has further divided ST131 into three main clades (A, B, and C), distinguished by variations in the fimH gene encoding type 1 fimbriae. Clade A typically carries fimH41, clade B fimH22, and clade C fimH30. Differences in fimH alleles likely influence adhesion and colonization capabilities among the clades. Within clade C, the presence of CTX-M-15 is linked to its subdivision into C1 and C2 subclades, a genetic adaptation that has facilitated the global spread of CTX-M-15-producing ST131 strains across both community and hospital settings.

Comprehensive molecular analyses have shown that the global spread of KPC-producing Enterobacteriaceae is largely driven by the clonal expansion of Klebsiella pneumoniae sequence type (ST) 258, a dominant lineage within clonal complex 258 (CC258). ST258 comprises two distinct clades, which differ primarily in their capsule polysaccharide biosynthesis gene regions. Interestingly, these clades display distinct genetic associations: clade I typically carries the blaKPC-2 gene, while clade II harbors blaKPC-3.

In contrast, the dissemination of NDM-producing Enterobacteriaceae results mainly from the horizontal transfer of broad-host-range plasmids carrying the blaNDM gene. Whole-genome sequencing has revealed that these plasmids belong to multiple incompatibility (Inc) groups—including IncA/C, IncF, IncH, IncL/M, IncN, and IncX—and that the blaNDM gene is often embedded within highly variable flanking regions, reflecting its mobility among nonclonal bacterial isolates.

Similarly, IMP-type carbapenemase genes are typically located within gene cassettes integrated into class 1 integrons, which are frequently associated with IncL/M and IncA/C plasmids. VIM-type genes also reside within class 1 integrons and may integrate into either plasmids or chromosomal DNA. In K. pneumoniae, the blaVIM-1 and blaVIM-4 genes are commonly located on N-type plasmids, whereas in E. coli, they are often found on IncFI/II plasmids.

Unlike other carbapenemase genes, the dissemination of OXA-48-type β-lactamases is largely associated with a single plasmid lineage—IncL/M—carrying the transposon Tn1999. This stable plasmid-mediated vehicle has been instrumental in the rapid interspecies and interregional spread of OXA-48-producing Enterobacteriaceae across healthcare settings.

4. β-Lactamase-Mediated Resistance in *Enterobacteriaceae*: Clinical Importance

Over the past two decades, β-lactam resistance among Enterobacteriaceae has risen sharply, accompanied by corresponding increases in morbidity, mortality, and healthcare costs. In Europe, deaths associated with third-generation cephalosporin-resistant E. coli and K. pneumoniae—the majority of which are ESBL producers—grew significantly between 2007 and 2015. A key contributor to this mortality burden is the delay in initiating appropriate antimicrobial therapy, often due to the time required for laboratory confirmation of resistance and the absence of rapid diagnostic tools.

Infections caused by ESBL-producing Enterobacteriaceae (ESBL-E) are associated with a marked increase in hospital length of stay (LOS) and treatment costs. Patients with bloodstream infections (BSIs) caused by ESBL-E experience longer hospitalizations—both in general wards and intensive care units—than those infected with non-ESBL-producing strains. Even noninvasive infections due to ESBL-E correlate with prolonged LOS and higher mortality rates.

The growing prevalence of ESBL-E has led to the widespread use of carbapenems as first-line therapy, which, while effective, poses a long-term risk of promoting carbapenem resistance. Treatment costs are substantially higher for ESBL-E BSIs compared with non-ESBL infections, reflecting both drug expense and prolonged care needs.

The use of β-lactam/β-lactamase inhibitor combinations (BL/BLIs)—notably piperacillin/tazobactam—for treating ESBL-E infections remains controversial. Clinical studies involving patients with ceftriaxone-resistant E. coli or K. pneumoniae BSIs have failed to demonstrate that piperacillin/tazobactam is non-inferior to carbapenems such as meropenem in terms of 30-day mortality. However, pooled analyses and meta-studies suggest that, in certain cases, BL/BLIs may offer outcomes comparable to carbapenems, supporting their role as viable carbapenem-sparing options in empiric or targeted therapy.

Recent clinical experiences have also highlighted the efficacy of ceftolozane/tazobactam, with multicenter studies reporting high clinical success rates in severe ESBL-E infections. Newer carbapenem-sparing regimens, such as ceftazidime/avibactam, have shown promising activity against ESBL-E isolates, although limited sample sizes have thus far precluded definitive conclusions.

As with ESBLs, the incidence, mortality, and economic burden of infections caused by carbapenemase-producing Enterobacteriaceae (CPE)—particularly K. pneumoniae—have increased significantly. The proportion of disability-adjusted life years (DALYs) attributable to carbapenem-resistant bacteria rose markedly during 2007–2015, with K. pneumoniae and E. coli accounting for an increasing share.

Globally, CPE infections are associated with high rates of clinical failure and a 20–40% mortality range. Meta-analyses confirm that infections due to carbapenem-resistant strains carry a substantially higher mortality risk compared with carbapenem-susceptible counterparts, especially in bloodstream infections and those caused by K. pneumoniae. Once again, delays in appropriate therapy and empiric treatment failures emerge as key predictors of poor outcomes.

Recent progress in antimicrobial development has yielded novel β-lactam/β-lactamase inhibitor combinations targeting carbapenemase-producing strains. These include ceftazidime–avibactam, meropenem–vaborbactam, and imipenem–relebactam, all demonstrating substantial efficacy and improved safety compared with older agents such as polymyxins.

Among these, ceftazidime–avibactam has shown superior outcomes in the treatment of KPC-producing infections, outperforming polymyxin-based regimens in both efficacy and tolerability. Imipenem–relebactam also offers a favorable clinical profile for infections caused by imipenem-nonsusceptible Enterobacteriaceae.

However, these drugs are ineffective against metallo-β-lactamase (MBL)-producing CPEs, such as NDM-, VIM-, or IMP-type enzymes. For these pathogens, polymyxins remain among the few remaining treatment options, albeit with recognized toxicity concerns. Promisingly, the new siderophore cephalosporin cefiderocol demonstrates potent activity against a wide range of class A, B, C, and D β-lactamases, including MBL-producing strains, and may represent a critical tool in the management of multidrug-resistant Enterobacteriaceae infections

Carbapenem-resistant Enterobacteriaceae (CRE), particularly carbapenem-resistant Klebsiella pneumoniae, impose a substantially greater economic burden on healthcare systems compared to other multidrug-resistant pathogens. These infections are associated with markedly higher direct medical costs, driven by extended hospital stays, complex treatment regimens, and the frequent need for intensive care management. Such data underscore that CRE infections are not only a clinical challenge but also a serious economic and logistical threat to the sustainability of healthcare systems. Consequently, international guidelines emphasize that carbapenems should be reserved strictly for cases with no viable alternative therapies, reinforcing the importance of antimicrobial stewardship and strict infection-control practices to curb their further emergence and dissemination.

5. Conclusions

Infections caused by extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) and carbapenem-resistant Enterobacteriaceae (CRE) continue to rise worldwide, contributing to escalating morbidity, mortality, and treatment costs. The increasing prevalence of these resistant organisms has compelled clinicians to

resort to combination regimens often involving older or more toxic agents, such as polymyxins or aminoglycosides. While these drugs can occasionally provide life-saving efficacy, their expanded use paradoxically drives further antimicrobial resistance and amplifies the economic burden on healthcare systems.

To address this growing crisis, a comprehensive understanding of the molecular mechanisms underlying β-lactamase-mediated resistance—and their ongoing evolutionary dynamics—is essential. This knowledge enables the targeted development of next-generation antimicrobial agents, optimizes infection-control interventions, and guides global and national health policies.

Equally critical is ensuring open access to current, region-specific data on resistance rates and molecular epidemiology. Such transparency empowers clinicians to make informed therapeutic choices, strengthens antibiotic stewardship programs, and facilitates international collaboration in monitoring and combating antimicrobial resistance. Ultimately, only a coordinated effort—combining molecular research, responsible drug use, and robust infection-control practices—can mitigate the threat posed by β-lactamase-producing Enterobacteriaceae and safeguard the efficacy of existing and future antimicrobial therapies.

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