



Targeted mechanisms and novel therapeutic strategies against extended-spectrum beta-lactamases: From precise detection to intelligent management of bacterial resistance

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ABSTRACT

Article info:

Received: 7 Aug 2025

Accepted: 22 Sep 2025

Keywords:

Antibiotic resistance

Bacterial infections

Beta-lactamase

Epidemiology

Diagnosis

Extended-spectrum beta-lactamases (ESBLs) are enzymes that resist beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, and are produced by a variety of bacteria, including *Escherichia coli* and *Klebsiella pneumoniae*. Since ESBLs are often plasmid-borne, antibiotic resistance can spread across bacteria due to their ease of transfer. The intricate structure of ESBLs changes based on the type of bacterium that produces them. However, they all share a beta-lactamase core structure. ESBLs act by hydrolyzing the beta-lactam ring of antibiotics thereby rendering them ineffective. Detection of ESBL-producing bacteria is very important for effective treatment of infections. These enzymes can be identified through various diagnostic methods, such as phenotypic tests and molecular assays. The most common diagnostic method is an antimicrobial susceptibility test, which involves testing bacterial sensitivity to different antibiotics. Furthermore, the use of molecular testing techniques like polymerase chain reaction is growing in the identification of ESBLs. This paper provides a summary of ESBLs, including their structure, function, and diagnostic methods. Thus, it is critical to comprehend ESBLs in order to create therapies that effectively address illnesses brought on by bacteria that produce ESBL.

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1. Introduction

Antibiotics from the beta-lactam family are some of the most widely used and efficient medications for the treatment of infectious diseases. Regrettably, microorganisms now have a variety of defensive mechanisms against these medications. One of the primary mechanisms of resistance is the production of beta-lactamase, which hydrolyzes the beta-lactam ring and renders the medication inactive [1]. Beta-lactamase enzymes hydrolyze cephalosporins, penicillins, monobactams, and carbapenems, thereby inactivating them. Extended-spectrum beta-lactamases (ESBLs) were discovered in the early 1980s and are a novel class of enzymes that hydrolyze penicillins and extended-spectrum cephalosporins. ESBLs are a group of enzymes that were primarily identified in members of the *Enterobacteriaceae* family, namely *Klebsiella pneumoniae*, isolated from patients hospitalized in special care units and later in the community. Additionally, certain Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, have also been shown to produce these enzymes [2-4]. Antimicrobial resistance in *Escherichia coli* has increased recently as a result of the careless and indiscriminate use of beta-lactam antibiotics. ESBL production by *E. coli* is considered a major concern in both human and animal populations because it leads to infections that are difficult to treat [5,6]. ESBLs have several classification types, the most common of which globally are Cefotaximase (CTX-M), Sulfhydryl variable (SHV), and Temoneira (TEM) [7].

The aim of this study is to review and evaluate the characteristics, roles, and detection methods of ESBL enzymes produced by bacteria. This research investigates the resistance mechanisms these enzymes employ against antibiotics and emphasizes the importance of their rapid detection for effective treatment of infections. Furthermore, the study addresses the obstacles and challenges in controlling antibiotic resistance and contributes to improved management of diseases caused by resistant bacteria by providing comprehensive knowledge on the structure, classification, and diagnostic techniques of ESBLs.

2. The evolution and genetic diversity of ESBLs

Beta-lactams account for 60% of all antibiotics produced and are the most widely used class of antibiotics due to their high tolerability and broad spectrum of activity [8,9]. Enzymes known as ESBLs are encoded by chromosomal and plasmid-borne genes and are produced by pathogens that exhibit resistance to aztreonam, penicillins, and oxyimino-cephalosporins. These enzymes are inhibited by clavulanic acid [10]. The most prevalent ESBL genes in human and animal Gram-negative bacteria include *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{OXA}, and *bla*_{SHV}, primarily carried by *E. coli* and *K.*

pneumoniae in healthcare settings. The emergence and spread of these genes represent a major concern in antimicrobial resistance [11,12]. The pharmacokinetic/pharmacodynamic (PK/PD) indices determine the bactericidal activity of beta-lactams [13]. The Ambler classification system classifies beta-lactamases into four classes based on amino acid sequence: class A (serine penicillinases), class B (metallo-beta-lactamases), class C (cephalosporinases), and class D (oxacillinases). Antibiotic resistance is mainly caused by a group of beta-lactamase-producing genes that include ESBLs, AmpC beta-lactamases, and carbapenemases [14]. In the Bush-Jacoby-Medeiros classification system, beta-lactamases are divided into three groups: cephalosporinases, serine beta-lactamases, and metallo- β -lactamases. However, the Ambler system is more widely used due to its simplicity [15]. Epidemiological studies indicate that the prevalence of ESBL-producing bacteria such as *K. pneumoniae* and *E. coli* is substantially higher in Asia compared to other regions globally, with China exhibiting the highest rate of ESBL production in *E. coli* isolates worldwide [16]. CTX-M beta-lactamases are the most widespread type, with their genes originating from the chromosome of genus *Kluyvera*. This genetic diversity enables hydrolysis of a broader range of cephalosporins. Although early CTX-M variants primarily hydrolyzed cefotaxime, over 60% of current isolates now also possess the ability to efficiently hydrolyze ceftazidime [17]. Among global ESBL isolates, CTX-M-2, CTX-M-3, and CTX-M-14 enzymes are the most prevalent, particularly among cephalosporin-resistant *K. pneumoniae* and *E. coli* strains [18]. TEM beta-lactamase is a key enzyme responsible for up to 90% of ampicillin resistance in *E. coli*, functioning through hydrolysis of the beta-lactam ring. This enzyme has evolved via point mutations at specific amino acid residues, with over 170 variants reported to date. While TEM confers resistance to penicillins and early cephalosporins, it can be inhibited by clavulanic acid. Global studies indicate that the *bla*_{TEM} gene is present in approximately 73% of ESBL-positive samples, with bacteriophages playing a significant role in its transmission [19-21]. SHV enzymes, encoded by the *bla*_{SHV} gene, are key mediators of penicillin resistance. The type 2b variants hydrolyze penicillins and cephalosporins, whereas types 2be and 2br have developed resistance to inhibition by clavulanic acid and tazobactam [17,22]. Oxacillinases (OXA), classified as class D carbapenemases, are frequently isolated from *A. baumannii*. This group is largely resistant to inhibition by clavulanic acid, a common characteristic. Although resistance to ceftazidime is rare among OXA enzymes, the OXA-17 variant exhibits greater resistance to cefixime and cefotaxime [23].

Rare ESBL variants such as integron-borne cephalosporinase-1 (IBC-1), Tlaloc-1 (TLA-1), Brazil extended-spectrum β -lactamase-1 (BES-1), and Serratia fonticola-1 (SFO-1) have been identified only in certain

members of the *Enterobacteriaceae* family. Research on other ESBLs has primarily focused on specific isolates of *P. aeruginosa* from limited geographic regions [24].

3. Mechanism of action of beta-lactam antibiotics

Mechanisms of antibiotic resistance include hydrolysis, redox processes, chemical group transfer, and modification of antibiotic target molecules. Hydrolytic enzymes can disrupt antibiotic activity by targeting and cleaving chemical bonds, while redox processes involve the oxidation or reduction of antibiotics [25]. Beta-lactam antibiotics inhibit bacterial transpeptidases, known as penicillin-binding proteins (PBPs), thereby preventing the cross-linking of peptidoglycan in the cell wall. By compromising PBPs, these antibiotics weaken the bacterial cell wall, leading to impaired growth and division, and reduced resistance to environmental stresses. The inhibitory activity of penicillin and other beta-lactam antibiotics is based on structural, geometric, and stereochemical similarities [26,27]. The target of these antibiotics is peptidoglycan, a macromolecule that covers the cytoplasmic membrane and provides structural stability to bacteria. Peptidoglycan is composed of glycan chains made of two alternating amino sugars, N-acetylglucosamine and N-acetylmuramic acid (NAM). These linear chains are cross-linked to each other by short peptide chains [28,29]. Because the beta-lactam ring mimics the structure of the D-alanine-D-alanine terminus of the

peptidoglycan peptide chain, beta-lactam antibiotics bind to PBPs. Beta-lactam antibiotics bind irreversibly to PBPs by mimicking this peptide structure, blocking their transpeptidase activity and preventing the formation of new cross-links in the peptidoglycan layer. This inhibition suppresses new cell wall formation, thereby weakening the existing bacterial cell wall. At the same time, autolysis of peptidoglycan takes place. This degradation leads to increased permeability and ultimately cell lysis [30,31] (Figure 1).

4. Diagnostic approaches to ESBLs identification

Methods for detecting beta-lactamase enzymes can be divided into phenotypic and genotypic categories. Phenotypic methods include double-disk synergy, gradient strip testing, and broth dilution [15]. These methods, particularly for ESBL identification, work by detecting synergistic interactions between beta-lactam drugs and specific enzyme inhibitors [32]. The combined disk test, typically performed using the disk diffusion method, is important for the accurate detection of ESBL enzymes in clinical laboratories. A key disadvantage of this method, however, is its lack of potential for automation. The Clinical and Laboratory Standards Institute (CLSI) has established the disk diffusion method as the standard for identifying ESBL production in *E. coli*, *K. pneumoniae*, and *K. oxytoca* [33]. Furthermore, the CLSI has developed a dilution-based antimicrobial susceptibility test to screen for ESBL production in *E. coli* and *Klebsiella* spp.

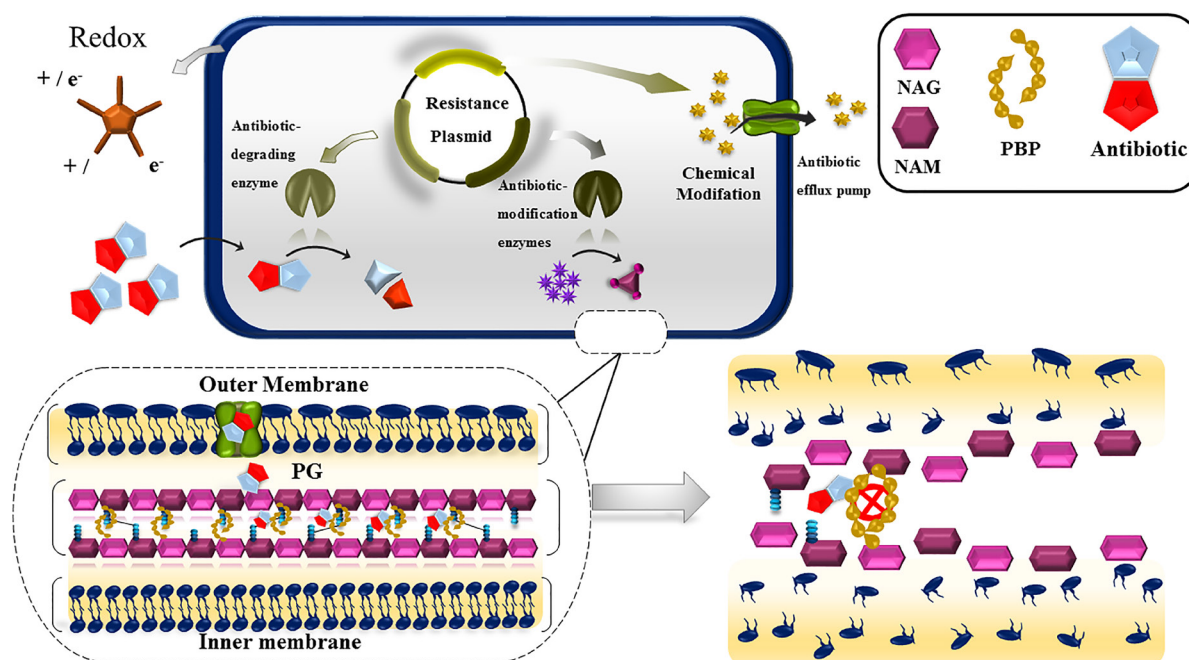


Figure 1. Mechanism of action of beta-lactam antibiotics and bacterial resistance via ESBL production. Beta-lactam antibiotics (e.g., penicillins, cephalosporins) inhibit bacterial cell wall synthesis by binding to PBPs, preventing cross-linking of peptidoglycan strands and leading to cell lysis. ESBLs confer resistance by hydrolyzing the beta-lactam ring, rendering the antibiotics ineffective. (NAM: N-acetylmuramic acid, NAG: N-acetylglucosamine, PG: Peptidoglycan).

This method uses ceftriaxone, ceftazidime, aztreonam, or cefotaxime at a screening concentration of 1 µg/ml [34-36]. Thin-layer chromatography is another rapid and simple technique for detecting beta-lactamases in clinical samples and evaluating their activity against various beta-lactam antibiotics, including carbapenems [37]. For confirmation, genotypic analysis of potential ESBL-producing strains is a promising alternative [38]. Molecular assays, such as polymerase chain reaction (PCR), real-time PCR, and microarrays, can identify carbapenemase-encoding genes. However, their dependence on expensive equipment and specialized technical expertise limits their adoption in resource-limited settings, particularly in low- and middle-income countries [39].

5. Emerging therapies and future prospects for overcoming ESBLs resistance

Carbapenems are the mainstay of treatment for infections caused by ESBL-producing bacteria. Intravenous administration is more effective than oral, but misuse and overuse have contributed to increased carbapenem resistance [40]. The US Food and Drug Administration has approved four drug combinations for clinical use against certain infections caused by Gram-negative pathogens: ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam, and imipenem-cilastatin-relebactam [41]. Additionally, new beta-lactamase inhibitors in clinical use include cefepime/enmetazobactam, aztreonam/avibactam, and sulbactam/durlobactam. Among these, cefepime/enmetazobactam is particularly effective against *P. aeruginosa* and ESBL-producing *Enterobacteriaceae* [42]. Nanotechnology, clustered regularly interspaced short palindromic repeats (CRISPR) systems, and phage therapy are examples of emerging technologies that address the key limitations of conventional antibiotics while enhancing traditional discovery approaches [43].

5.1 Nanotechnology-based approaches

Nanoparticles (or nanobiotics), owing to their unique electrical, magnetic, and binding properties, represent a promising strategy for fighting resistant infections. They function either directly as nanobactericides or as smart nanocarriers for the targeted delivery of established antibiotics [44]. Nanoantibiotics (nanoparticles with inherent antibacterial activity) improve the efficacy and safety of antibiotic therapies. Their advantages include an increased drug half-life, sustained effective concentration at the target site, a reduced dosing frequency, and minimized side effects [45].

Nanoparticles such as gold nanoparticles are emerging as an advanced and novel approach to treating infections caused by ESBL-producing bacteria. Due to their ability to deliver antibiotics specifically to resistant

strains and their multiple antibacterial mechanisms like biofilm inhibition, cell wall disruption, and induction of oxidative stress, they represent a promising strategy for combating antibiotic resistance [46].

5.2 Phage therapy

Phage therapy has also been extensively studied as a treatment for infections caused by antibiotic-resistant bacteria and is considered a promising alternative to traditional therapies for this major public health challenge. This approach is rapidly gaining attention in research [47]. Bacteriophages are diverse and abundant viruses with effective bactericidal properties. According to a recent study, the lytic bacteriophages EC.W1-9 and EC.W15-4 show high therapeutic potential against the global threat of ESBL-producing *E. coli* isolates [48].

Phages attach to specific receptors on bacterial cells, inject their genetic material, and initiate a lytic replication cycle. They can also integrate their genetic material into the bacterial genome, replicating as the bacterial cell divides [49]. For therapeutic research and clinical applications, non-lysogenic phages and wild-type or engineered lytic phages are employed. The emergence of strains resistant to both phages and antibiotics is mitigated through phage-antibiotic therapy [50].

Moreover, to counter phage-resistant bacteria, the use of phage cocktails is essential. However, before this approach can be widely adopted in therapy, several challenges must be addressed, including the limited host range of phages, systemic side effects, phage resistance, rapid clearance of phages by the immune system, issues in phage delivery, and challenges in phage production [51].

5.3 CRISPR-Cas systems

The CRISPR-Cas system is an adaptive immune system found in bacteria and archaea. In 2012, it was demonstrated that the Cas9 protein could be programmed to cleave DNA at specific sites, transforming it into a precise tool for genome editing in agriculture and medicine. This system holds the potential to target genetic resistance determinants, offering a precise alternative for controlling Gram-negative bacteria [52]. The number of CRISPR loci per genome can vary, but the most effective system is CRISPR-Cas9, as it utilizes all major biological molecules: protein, RNA, and DNA and offers greater customizability and flexibility [53].

Differences in CRISPR loci among bacterial species influence the efficacy of CRISPR-Cas systems. To ensure the safety and precision of these systems in clinical settings, issues such as improving delivery strategies and mitigating off-target effects must still be resolved [54]. Several studies have demonstrated that CRISPR-Cas systems can be engineered into probiotic

bacteria to specifically target and destroy pathogenic or drug-resistant bacteria or eliminate antimicrobial resistance genes [55].

The CRISPR-Cas targeting system effectively combats antibiotic resistance by selectively inactivating genes involved in biofilm formation, pathogenesis, virulence, or bacterial survival through chromosomal gene cleavage or plasmid elimination [56]. Furthermore, the CRISPR-Cas system is used alongside other tools to enable easier, more specific, and more sensitive detection of bacterial infections from samples [57].

6. Conclusion

Today, we are witnessing increasing bacterial resistance and infections caused by them in the world. One of the most pressing issues in the management of illnesses brought on by these bacteria is this issue. The rapid transmission and spread of organisms capable of producing the aforementioned enzymes has caused an increase in hospital-related infections around the world. ESBL has grown a lot over the past years. Its generating genes can be spread between bacteria through plasmid transfer.

Researchers face challenges in detecting ESBLs in clinical microbiology labs, requiring control of drug use, monitoring of treatment strains, enzyme detection tests, and the use of appropriate antibiotics to prevent resistance. Therefore, combating ESBL and other antimicrobial resistance requires adopting novel strategies including next-generation beta-lactamase inhibitors, nanoparticle-based therapies, engineered CRISPR-Cas systems, and phage therapy. These approaches show significant promise but demand rigorous research and innovative implementation to mitigate the global burden of drug-resistant infections and improve treatment outcomes.

Authors' contributions

AJS, MP: Conceived the main idea of the manuscript, edited drafts. AG, ZG: Literature search and manuscript preparation. AG, ZG: Figure design. AJS, AG, ZG, MSF, MP, AAZD: Data collection and drafting the manuscript. All authors read and approved the final manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

Ethical declarations

Not applicable.

Financial support

Self-funded.

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