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Review Article

Mechanisms of Antimicrobial Resistance in Gram-Negative Bacteria: A Review of β-Lactamases, Efflux Pumps, and Horizontal Gene Transfer

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ABSTRACT
Antimicrobial resistance (AMR) is a major global health problem that makes many common bacterial infections harder to treat. This global problem causes longer hospital stays, higher treatment costs, and increased deaths worldwide. Gram-negative bacteria are especially important in this crisis because they can resist many antibiotics and spread easily in hospitals and communities. Although individual antimicrobial resistance mechanisms in Gram-negative bacteria are well characterized, the interactions and combined effects of multiple mechanisms, particularly their synergistic contributions to high-level resistance, remain incompletely understood. This mini narrative review examines three main resistance mechanisms in Gram-negative bacteria, namely β-lactamase enzymes, efflux pump systems, and horizontal gene transfer. A literature search was conducted in PubMed, Web of Science, Google Scholar and Scopus using the keywords "horizontal gene transfer", "Gram-negative bacteria", "β-lactamase," and "efflux pump" and boolen operators "AND, OR". Articles used were mostly publications between 2015 and 2025. The findings show that β-lactamases break down antibiotics such as penicillin and carbapenems, Efflux pumps reduce antibiotic concentration inside bacterial cells by actively pumping drugs out, and horizontal gene transfer spreads resistance genes quickly through plasmids, transposons, and integrons. These mechanisms most of the time work in synchronization in the cell, creating a stronger and more complex multilayered resistance pattern, as explained in detail in the full review article below. Overall, antimicrobial resistance in Gram-negative bacteria is driven by multiple interacting systems rather than a single mechanism, highlighting the need for integrated diagnostic, treatment, and surveillance strategies to effectively control its global impact.

Keywords: β-lactamases; Antimicrobial resistance; Efflux pumps; Gram-negative bacteria; Horizontal gene transfer; Plasmids

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INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most urgent public health challenges of the twenty-first century, threatening the effective prevention and treatment of bacterial infections all over the world. According to the World Health Organization (WHO), bacterial AMR was directly responsible for approximately 1.27 million deaths globally in 2019 and indirectly contributed to about 4.95 million deaths (World Health Organization, 2023). This statistic clearly shows that AMR is not a future risk but an ongoing global health issue that is already causing substantial loss of life and placing serious pressure on healthcare systems.

AMR simply refers to the ability of microorganisms to survive and continue growing despite being exposed to antimicrobial agents that would normally kill them or stop them from growing. While resistance is a natural biological process that occurs as part of evolution in bacteria, its current acceleration is strongly linked to human activities. The overuse and misuse of antibiotics in human medicine, veterinary

practice, and agriculture have significantly increased the factors that cause resistant strains to emerge and spread (Muteeb *et al*., 2023; Petrosillo & Granata, 2022).

As a result, infections that were once easy to treat are becoming increasingly difficult to manage, leading to higher mortality rates and increased healthcare costs. Importantly, evidence from ancient and previously unstudied cases shows that antimicrobial resistance is not a new phenomenon. Studies have demonstrated that resistance genes existed in environmental microorganisms long before the introduction of modern antibiotics. (Petrosillo & Granata, 2022)

This indicates that bacteria naturally possess genetic systems capable of resisting antimicrobial compounds as part of their long-term evolutionary adaptation (Petrosillo & Granata, 2022). However, what distinguishes the current situation from historical patterns is the scale and speed at which resistance is spreading. Modern AMR is expanding far more rapidly and extensively than in the past, creating a global health threat that is significantly more severe and widespread than historical levels of bacterial resistance (Nwobodo *et al*., 2022).

Among the diverse groups of bacteria involved in this crisis, Gram-negative bacteria represent a particularly important and concerning type. These organisms, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are frequently implicated in both community-acquired and hospital-acquired infections such as urinary tract infections, bloodstream infections, pneumonia, and wound infections (Ndung'u, 2024; Wang *et al*., 2021). Their clinical importance is further amplified by their high capacity to develop and spread multidrug resistance, often leading to treatment failure even with commonly used antibiotics.

Gram-negative bacteria are especially difficult to treat because of their unique structural and biological characteristics (Lepe & Martínez-Martínez, 2022). They have an outer membrane that serves as a protective barrier that limits antibiotic entry which reduces drug effectiveness. In addition, these bacteria possess a diverse array of adaptive resistance mechanisms, including enzymatic degradation of antibiotics, active efflux systems, and the acquisition of resistance genes through horizontal gene transfer (Lepe & Martínez-Martínez, 2022).

Mobile genetic elements such as plasmids further enhance this process by enabling the rapid transfer of resistance genes between bacteria, both within and across species (Larsson & Flach, 2022). Together, these features create highly resilient pathogens capable of surviving in diverse clinical environments.

Despite significant advances in antimicrobial research, a major gap still exists between the rapid emergence of resistance and the development of effective therapeutic solutions. The discovery of new antibiotics has slowed down considerably because of scientific, economic, and policy challenges, while resistance continues to evolve at a faster rate than the development of new drugs (Muteeb *et al*., 2023).

More importantly, the interaction between different resistance mechanisms within Gram-negative bacteria is still not fully understood. Most existing studies tend to examine β-lactamases, efflux pumps, and horizontal gene transfer separately, without fully exploring how these systems interact and reinforce each other in realistic healthcare settings. This limits the development of comprehensive strategies to effectively control multidrug-resistant infections.

Understanding these mechanisms in an integrated manner is therefore essential for addressing the AMR crisis. Resistance in Gram-negative bacteria is not the result of a single process but rather a complex system of interconnected biological strategies that collectively enhance survival and adaptation. By studying these systems together, it becomes possible to better understand how resistance develops, spreads, and persists in the hospital and in the general community.

This review integrates three interconnected major resistance mechanisms of antimicrobial resistance in Gram-negative bacteria: β-lactamase production (enzymatic degradation), efflux pump systems (active drug extrusion), and horizontal gene transfer (genetic exchange)-to simplify and explain how Gram negative bacteria achieve multidrug resistance.

MATERIALS AND METHODS

This review was conducted following the preferred reporting items for mini narrative reviews and guidelines. A systematic literature search was performed in four electronic databases: PubMED, Web of Science, Google Scholar and Scopus. The search terms used were: ("Gram-negative bacteria" OR "*Enterobacterales"* OR "*Pseudomonas aeruginosa*" OR "*Acinetobacter baumannii")* AND ("antimicrobial resistance" OR "antibiotic resistance") AND ("β-lactamases" OR "ESBL" OR "carbapenemase") AND ("efflux pumps" OR "AcrAB" OR "MexAB") AND ("horizontal gene transfer" OR "plasmids" OR "integrons" OR "transposons") were used to combine terms.

Inclusion criteria:
(1) All original research articles, systematic reviews, and meta-analyses published between 2015 and 2025; this was chosen because we want to analyze only recent work and development in the area of AMR in Gram negative bacteria.
(2) Most studies reporting on resistance mechanisms in Gram-negative bacteria;
(3) Only articles written in English language.
Exclusion criteria:
(1) Conference abstracts, editorials, and opinion pieces;
(2) Studies focusing only on Gram-positive bacteria;
(3) Articles without full-text access.
Titles and abstracts were screened, followed by full-text review. The quality of included studies was assessed using the Joanna Briggs Institute (JBI) critical appraisal checklists for systematic reviews. Data were extracted into a standardized form and synthesized narratively.
Section 2 discusses the molecular mechanisms of resistance, beginning with β-lactamases, followed by efflux pumps, and then horizontal gene transfer.
Section 3 examines the interaction between these mechanisms, their geographical distribution, and the challenges associated with detection and diagnosis. Finally, Section 4 synthesizes the findings and presents conclusions and future perspectives, highlighting key gaps and proposing potential strategies for addressing antimicrobial resistance in Gram-negative bacteria.
In summary, AMR in Gram-negative bacteria represents a complex and rapidly evolving global health threat. A deeper understanding of its underlying mechanisms and their interactions is essential for developing more effective diagnostic, therapeutic, and preventive strategies.

RESULTS

β-Lactamase Enzymes

β-lactamase enzymes are bacterial proteins that offer resistance by hydrolyzing the β-lactam ring, an important part of the structure for penicillins, cephalosporins, monobactams, and carbapenems. This hydrolysis renders the antibiotic molecule inactive before it can bind to penicillin-binding proteins, effectively neutralising its bactericidal activity. β-lactamases are among the most clinically important resistance determinants in Gram-negative bacteria because they are often carried on plasmids and can spread rapidly across species through horizontal gene transfer. Their diversity and adaptability have contributed significantly to the global burden of multidrug-resistant infections (Queenan *et al*., 2007; Castanheira *et al*., 2021).

Classification (Ambler classes A–D)

β-lactamases are classified into four Ambler classes (A–D) based on their amino acid sequence and catalytic mechanism. Class A, C, and D enzymes have active sites that uses serine to break down antibiotics, while class B enzymes are metallo-β-lactamases that require zinc ions for activity. Class A includes extended-spectrum β-lactamases (ESBLs) such as TEM, SHV, and KPC carbapenemases. Class B includes metal-seeking enzymes such as NDM, VIM, and IMP, which have broad activity against carbapenems. Class C enzymes are primarily AmpC β-lactamases, which used to be originally found in the main bacterial DNA but now often carried on plasmids. Class D enzymes include OXA-type β-lactamases, particularly OXA-48-like carbapenemases, which are widespread in Enterobacterales. These classes differ in substrate specificity, inhibitor susceptibility, and clinical impact, but collectively contribute to broad-spectrum resistance and treatment failure in Gram-negative infections (Queenan *et al.,* 2007; Han *et al*., 2020).

ESBLs (CTX-M, SHV, TEM)

Extended-spectrum β-lactamases (ESBLs) are primarily derived from TEM, SHV, and CTX-M families and are highly resistant to third-generation cephalosporins and monobactams. Historically, TEM and SHV enzymes dominated ESBL production; however, CTX-M-type enzymes have now become a global phenomenon, with CTX-M-15 being the most widespread variant (Castanheira *et al*., 2021). ESBL genes are typically found on plasmids and often embedded within transposons and insertion sequences, which facilitates rapid dissemination across Enterobacterales. ESBL-producing *Escherichia coli*, particularly sequence type ST131, has emerged as a dominant global clone associated with both community and hospital infections. These enzymes do not typically hydrolyse carbapenems, but their presence often leads to increased carbapenem use, which in turn accelerates selection pressure for carbapenemase-producing strains. Clinically, ESBLs are associated with delayed effective therapy and increased reliance on last-line antibiotic solutions such as carbapenems, contributing indirectly to global resistance escalation (Castanheira *et al.,* 2021).

Carbapenemases (KPC, NDM, OXA-48)

Carbapenemases are β-lactamases capable of hydrolysing carbapenems, representing a critical escalation in antimicrobial resistance. The most clinically important carbapenemases include KPC (class A), NDM (class B), and OXA-48-like enzymes

(class D). KPC enzymes are widely disseminated in *Klebsiella pneumoniae* and are often associated with hospital outbreaks. NDM enzymes, first identified in the Indian subcontinent, are notable for their broad substrate profile and resistance to most β-lactams except aztreonam. OXA-48-like enzymes are particularly concerning because they often offer only low-level carbapenem resistance in vitro, making detection difficult despite their strong clinical association with treatment failure (Han *et al*., 2020). These enzymes are frequently carried on highly mobile plasmids such as *IncL* and *IncF* types, which enhance their global dissemination. Collectively, carbapenemases are very difficult to treat because there are few drug options and bacteria keep changing quickly (Lee *et al*., 2022).

Epidemiology and detection

The epidemiology of β-lactamases is characterized by rapid global dissemination driven by plasmids, creation of multiple clones, and transmission in healthcare settings. ESBLs are now easily found in both hospital and community settings worldwide, while carbapenemases such as KPC and NDM are increasingly prevalent in Asia, Europe, and Latin America. OXA-48-like enzymes are highly prevalent in Europe, North Africa, and the Middle East, often remaining underdiagnosed due to weak phenotypic expression (Boyd *et al.,* 2022). Detection remains challenging because standard susceptibility testing may fail to identify low-level resistance or masked resistance mechanisms. Phenotypic methods such as combination disk tests and Carba NP assays are widely used, but molecular methods including PCR and whole-genome sequencing offer higher accuracy. However, resource limitations in many settings hinder widespread molecular surveillance. This diagnostic gap contributes to underestimation of the true burden of carbapenemase-producing Enterobacterales and delays appropriate therapy, worsening clinical outcomes (Imkamp *et al*., 2022; Lee *et al.,* 2022).

Efflux Pumps

Althoug β-lactamase enzymes directly break down antibiotics through the process of enzymatic degradation, Gram-negative bacteria also use other methods to lower the amount of drug inside the cell without actually destroying it. One of the most significant of these systems involves membrane-associated transport proteins known as efflux pumps, which actively export a wide range of antimicrobial compounds. This section examines these efflux systems in detail, highlighting their structure, substrate specificity, and role in both intrinsic and acquired resistance, as well as their clinical implications in multidrug-resistant infections. Efflux pumps are membrane-associated transport systems that actively expel toxic compounds, including antibiotics, from bacterial cells. Unlike β-lactamases, which chemically inactivate antibiotics, efflux pumps reduce intracellular drug concentration below therapeutic levels, allowing bacteria to survive even in the presence of active antimicrobial agents. These systems are a key component of intrinsic resistance in Gram-negative bacteria and also contribute significantly to acquire multidrug resistance when overexpressed or combined with horizontally acquired resistance genes. Efflux activity is particularly important in organisms such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii*, where it works together with a strong outer membrane and enzymatic degradation to make create a highly resistant variant of the bacteria (Lorusso *et al*., 2022; Laborda *et al.,* 2024).

RND family (AcrAB-TolC, MexAB-OprM)

The Resistance-Nodulation-Division (RND) family is the most clinically significant efflux pump system in Gram-negative bacteria. These are three-way protein systems that span the inner membrane, periplasm, and outer membrane, enabling direct export of antibiotics out of the cell. In Escherichia coli, the AcrAB-TolC system is the dominant efflux pump, while in *Pseudomonas aeruginosa,* MexAB-OprM serves a similar role. These systems consist of an inner membrane transporter, a periplasmic adaptor protein, and an outer membrane channel that together form a continuous efflux tunnel *t*hat pushes antibiotics out of the cell*.* RND pumps are energy-driven via proton motive force, allowing them to expel a wide variety of structurally unrelated compounds. Their broad substrate range and high efficiency make them central contributors to multidrug resistance and reduced susceptibility to multiple antibiotic classes (Jang, 2023; Lorusso *et al*., 2022).

Substrate profiles

RND efflux pumps exhibit exceptionally broad substrate specificity, enabling bacteria to resist multiple antibiotic classes simultaneously. Substrates include β-lactams, fluoroquinolones, tetracyclines, macrolides, chloramphenicol, and certain antiseptics and detergents. This ability to handle many drugs comes from large, flexible areas that can fit different types of chemicals. In Pseudomonas aeruginosa, different efflux systems (MexAB-OprM, MexXY-OprM, MexCD-OprJ) contribute distinct but

overlapping substrate profiles, creating redundancy that strengthens resistance. Similarly, AcrAB-TolC in E. coli can export both hydrophilic and hydrophobic antibiotics. Importantly, efflux pumps can also transport host-derived antimicrobial peptides and signaling molecules, linking resistance mechanisms to virulence and bacterial communication systems. This broad specificity ensures that inhibition of a single antibiotic class rarely restores full susceptibility, complicating therapeutic strategies (Laborda *et al.,* 2024; Lorusso *et al.,* 2022).

Role in intrinsic and acquired resistance

Efflux pumps contribute to both intrinsic and acquired resistance mechanisms. Intrinsic resistance arises from the baseline expression of efflux systems that low levels of antibiotics that don't fully stop bacteria*,* naturally reduce intracellular antibiotic accumulation, particularly in Gram-negative bacteria with restrictive outer membranes. Acquired resistance occurs when efflux pump genes are overexpressed due to mutations in regulatory genes or when global stress responses are activated under antibiotic pressure. Additionally, horizontal gene transfer can introduce regulatory elements that enhance efflux pump expression. In many bacteria samples extracted from patients, efflux pump over activity acts synergistically with β-lactamase production, creating high-level multidrug resistance. Importantly, resistance caused by pushing drugs out of the cell, can also promote persistence by enabling survival at thereby facilitating the selection of additional resistance mutations (Ding *et al*., 2023; Lorusso *et al*., 2022).

Clinical relevance

Clinically, efflux pumps are major contributors to treatment failure in infections caused by multidrug-resistant Gram-negative pathogens. Their ability to reduce intracellular antibiotic concentrations undermines the effectiveness of nearly all major antibiotic classes. In Pseudomonas aeruginosa, efflux overexpression is strongly associated with difficult-to-treat resistance phenotypes and increased mortality risk in hospital-acquired infections. Efflux pumps also contribute to infections where bacteria grow in protective layers (known as biofilms)*,* that limits drug penetration thereby further stopping antibiotics from working effectively. Importantly, efflux inhibitors such as carbonyl cyanide m-chlorophenyl hydrazone (CCCP), Phe-Arg-β-naphthylamide (PAβN), and 1-(1-naphthylmethyl)-piperazine (NMP) have demonstrated the ability to restore antibiotic susceptibility in experimental settings, highlighting their potential as supporting treatment solutions that are used together with main drugs*.* However, no clinically approved efflux pump inhibitors currently exist, largely due to toxicity and lack of specificity (Ding *et al.,* 2023; Laborda *et al.,* 2024).

Horizontal Gene Transfer

Horizontal gene transfer (HGT) is a fundamental evolutionary process in bacteria that enables the direct acquisition of genetic material from unrelated organisms. Unlike vertical inheritance, which passes traits from parent to offspring, HGT allows bacteria to rapidly gain adaptive functions such as antimicrobial resistance, virulence traits, and metabolic capabilities. This mechanism is central to the global spread of multidrug-resistant pathogens, particularly among Gram-negative bacteria where mobile genetic elements (MGEs) act as efficient vehicles for gene exchange. As highlighted in earlier sections, β-lactamases and efflux pump regulators are frequently mobilised through HGT, making it a key driver of resistance evolution in clinical environments (Arnold *et al*., 2022; Tokuda *et al*., 2024).

Conjugative plasmids (Inc groups)

Conjugative plasmids are the primary drivers of horizontal gene transfer in clinically relevant bacteria. These extrachromosomal DNA elements replicate independently and transfer between bacteria via direct cell-to-cell contact. They are classified into incompatibility (Inc) groups based on replication control systems, with IncF, IncL, IncHI, IncR, and IncX being particularly important in Enterobacterales. These plasmids frequently carry multiple antibiotic resistance genes, including ESBLs (CTX-M), carbapenemases (KPC, NDM, OXA-48-like), and genes encoding resistance to aminoglycosides and fluoroquinolones. Some plasmids also co-harbour virulence determinants, enhancing bacterial fitness and pathogenicity. Their ability to persist in bacterial populations without strong antibiotic pressure further enhances their epidemiological success. In *Klebsiella pneumoniae*, hybrid plasmids combining multiple replicons have been identified, demonstrating high structural plasticity and evolutionary adaptability (Long *et al*., 2023; Han *et al*., 2020).

Transposons and integrons

Transposons and integrons are key genetic elements that facilitate the rearrangement and capture of antibiotic resistance genes within bacterial genomes. Transposons are mobile DNA segments capable of moving between chromosomal and plasmid locations, often carrying resistance genes such as bla genes encoding β-lactamases. Integrons, on the other

hand, function as genetic platforms that capture gene cassettes through a controlled DNA insertion at specific spots. Class 1 integrons are particularly associated with clinical resistance and frequently contain groups of antibiotic resistance determinants. These systems do not move independently but are often embedded within plasmids or transposons, increasing their mobility. Together, they contribute to the assembly of multidrug resistance regions and accelerate the evolution of complex resistance phenotypes. Integrons also play a broader evolutionary role by acting as reservoirs of adaptive genes beyond antibiotic resistance, including stress response and survival traits (Tokuda *et al.,* 2024; Wachino, 2025).

Role of mobile genetic elements in spreading resistance genes

Mobile genetic elements are the principal vehicles for the dissemination of antibiotic resistance genes across bacterial populations and environments. Plasmids, transposons, integrons, bacteriophages, and more recently outer membrane vesicles collectively facilitate gene exchange at high frequency. These elements enable resistance genes to move not only between strains of the same species but also across different genera, accelerating global dissemination. In clinical settings, MGEs are responsible for the rapid emergence of carbapenem-resistant Enterobacterales and other multidrug-resistant organisms. Their role is further amplified by selective pressure from antibiotic use, which favours bacteria carrying transferable resistance determinants. Additionally, MGEs can integrate multiple resistance mechanisms into a single genetic platform, producing highly stable multidrug resistance clusters. This convergence of mobility and selection pressure explains the exponential spread of resistance traits in hospital and community environments (Tokuda *et al*., 2024; Wachino, 2025).

Co-transfer of resistance and virulence

A critical and emerging concern in bacterial evolution is the co-transfer of antimicrobial resistance and virulence genes on the same mobile genetic elements. Hybrid plasmids have been identified that simultaneously carry carbapenemase genes (such as blaOXA-181 or tet(X4)) and virulence operons like aerobactin (iutA). This genetic coupling enhances both survival under antibiotic pressure and pathogenic potential within the host. In *Klebsiella pneumoniae,* such plasmids can transform classical strains into hyper-virulent, multidrug-resistant variants capable of causing severe invasive infections. Importantly, these plasmids are often stable and transferable, even in the absence of antibiotic selection, increasing their epidemiological persistence. The convergence of resistance and virulence represents a significant evolutionary step that complicates infection control and treatment strategies, as it produces pathogens that are both highly transmissible and difficult to treat (Liu *et al.,* 2022; Han *et al*., 2025).

DISCUSSION

Synergy and Clinical Impact

The resistance landscape in Gram-negative bacteria cannot be understood through isolated mechanisms alone. As demonstrated in the preceding sections, β-lactamases, efflux pumps, and horizontal gene transfer systems operate simultaneously within the same bacterial populations. Their interaction produces multiple resistance mechanisms that working together whereby enzymatic drug destruction reduced intracellular drug accumulation and rapid genetic exchange reinforce each other. This convergence is what transforms individual resistance traits into clinically significant multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes. The interaction of multiple resistance mechanisms significantly complicates treatment outcomes, particularly in hospital-associated infections caused by *Enterobacterales, Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Tokuda *et al*., 2024

Co-occurrence of multiple mechanisms

In many bacteria samples extracted from patients, resistance mechanisms co-exist within a single bacterial cell, creating synergistic protection against antibiotics. For example, carbapenem-resistant Enterobacterales often produce β-lactamases such as KPC or NDM while simultaneously overexpressing efflux pumps and acquiring permeability defects. Similarly, Pseudomonas aeruginosa may combine intrinsic efflux activity (MexAB-OprM), porin loss, and inducible β-lactamase production. These combined mechanisms do not act independently; instead, they reinforce one another by reducing antibiotic entry, degrading any remaining drug, and expelling intracellular molecules that avoid being damaged by enzymes. This redundancy ensures survival even under high antibiotic concentrations. Additionally, mobile genetic elements facilitate the co-localisation of multiple resistance genes on the same plasmid, further enhancing co-selection under antibiotic pressure (Lorusso *et al*., 2022; Tokuda *et al*., 2024).

Treatment failures (carbapenem-resistant Enterobacteriaceae)

Carbapenem-resistant Enterobacterales (CRE) represent a major clinical challenge due to their association with high mortality and limited treatment options. Treatment failure is frequently linked to the combined presence of carbapenemases (e.g., KPC, NDM, OXA-48-like), efflux pump overexpression, and additional resistance determinants carried on plasmids. Clinical studies show that factors such as high disease severity scores, invasive devices, and infection with *Klebsiella pneumoniae* significantly increase the risk of poor outcomes (Rebold *et al*., 2023). Even with the introduction of novel β-lactam/β-lactamase inhibitor combinations such as ceftazidime-avibactam and meropenem-vaborbactam, resistance can emerge through plasmid evolution or enzyme mutation. In addition, heteroresistance and poor antibiotic penetration in biofilms further reduce therapeutic efficacy. As a result, CRE infections often require combination therapy, prolonged treatment courses, and careful and responsible use of antibiotics to improve clinical outcomes (Zhang *et al*., 2026; Kanj *et al*., 2022).

Challenges for susceptibility testing

Accurate detection of resistance mechanisms remains a significant challenge in clinical microbiology. Phenotypic susceptibility testing often fails to fully capture the complexity of resistance, particularly when enzymes confer only low-level resistance or when multiple mechanisms mask each other's effects. For instance, OXA-48-like carbapenemases may produce minimal changes in minimum inhibitory concentrations (MICs), leading to under-detection in routine testing. Similarly, efflux-mediated resistance and permeability defects are difficult to quantify using standard laboratory methods. Molecular techniques such as PCR and whole-genome sequencing improve detection accuracy but are limited by cost, infrastructure requirements, and turnaround time. Furthermore, the continuous emergence of new β-lactamase variants and mobile genetic elements complicates breakpoint interpretation and diagnostic standardization. These limitations contribute to delays in appropriate therapy, misclassification of resistance profiles, and underestimation of the true burden of antimicrobial resistance (Imkamp *et al*., 2022; Wenzler *et al.,* 2023).

Building on the preceding analysis, it is evident that antimicrobial resistance in Gram-negative bacteria is not driven by isolated mechanisms but by a coordinated and dynamic network of interacting processes. β-lactamases, efflux pumps, and horizontal gene transfer do not function independently; rather, they operate within an integrated system that enhances bacterial survival under sustained antimicrobial pressure. This section synthesizes these mechanisms to demonstrate how their interaction produces clinically significant resistance phenotypes, explores how these patterns vary across global regions, and critically evaluates the limitations of current detection strategies. Importantly, the findings discussed throughout this review converge on a central conclusion: resistance is best understood as a systems-level phenomenon shaped by both molecular interactions and epidemiological forces. This perspective is essential for interpreting clinical outcomes, guiding treatment decisions, and informing future research and formulation of guidelines and decisions.

Overlap and cooperation between mechanisms

The interaction between β-lactamases, efflux pumps, and horizontal gene transfer represents a key driver of multidrug resistance in Gram-negative bacteria. As established in Section 2, these mechanisms form a layered defence system in which each component compensates for the limitations of others. β-lactamases enzymatically inactivate antibiotics, efflux pumps reduce intracellular drug concentrations, and horizontal gene transfer continuously introduces and disseminates resistance determinants across bacterial populations. Their combined effect is not merely additive but synergistic, resulting in resistance levels that exceed the impact of individual mechanisms alone.

For instance, carbapenem-resistant Enterobacterales frequently co-express carbapenemases such as KPC or NDM alongside overactive efflux systems and reduced membrane permeability. This combination ensures that even if partial antibiotic activity is retained, drug concentrations within the cell remain insufficient to effectively kill bacteria (Lorusso *et al*., 2022; Tokuda *et al*., 2024). Similarly, in Pseudomonas aeruginosa, intrinsic efflux activity (e.g., MexAB-OprM) operates in parallel with inducible β-lactamase production and adaptive mutations, creating highly resilient resistance phenotypes (Letizia *et al*., 2025).

Crucially, horizontal gene transfer accelerates this synergy by enabling the co-localization of multiple resistance genes on the same mobile genetic elements. As a result, bacteria can rapidly acquire complex resistance profiles in a single genetic event, reinforcing the argument that resistance evolution is both cumulative and cooperative. These interactions

directly explain the persistence of multidrug-resistant infections despite aggressive antimicrobial therapy.

Geographic variation in dominant resistance types

While the underlying mechanisms of resistance are conserved, their distribution varies significantly across geographic regions, reflecting differences in antibiotic use, healthcare infrastructure, and surveillance capacity. Global surveillance data indicate that carbapenem resistance is widespread but unevenly distributed, with particularly high prevalence in parts of Asia, where pooled estimates reach approximately 31% (Jayathilaka *et al*., 2025).

Within these regions, metallo-β-lactamases such as NDM predominate, whereas KPC enzymes are more common in Europe and the Americas, and OXA-48-like enzymes are frequently reported in North Africa and the Middle East (Jean *et al*., 2025).

These regional differences highlight the role of horizontal gene transfer in shaping local resistance landscapes. The dissemination of specific plasmid types, such as *IncF* and *IncL*, facilitates the regional dominance of particular β-lactamase genes, while creation of multiple clones further reinforces their spread (Long *et al*., 2023). Despite this variability, the clinical consequences remain remarkably consistent: reduced antibiotic effectiveness, increased reliance on last-line treatment solutions, and elevated mortality rates.

Importantly, geographic variation does not imply the differences that the resistance works but rather reflects different combinations and prevalence of the same core resistance strategies. In this sense, resistance is globally heterogeneous at the genetic level but functionally convergent at the clinical level. This convergence underscores the need for region-specific surveillance combined with globally coordinated intervention strategies to effectively address antimicrobial resistance.

Limitations of current detection methods

The complexity and interaction of resistance mechanisms present significant challenges for accurate detection and clinical management. As discussed previously, phenotypic susceptibility testing often fails to capture the full extent of resistance, particularly when multiple mechanisms operate simultaneously or when resistance is expressed at low levels.

For example, OXA-48-like carbapenemases may produce minimal changes in susceptibility profiles, leading to under-detection despite their clinical significance (Imkamp *et al*., 2022). Similarly, resistance caused by drug-pumping systems and permeability defects are difficult to quantify using standard laboratory methods, further obscuring true resistance phenotypes.

Molecular approaches such as polymerase chain reaction and whole-genome sequencing offer improved sensitivity and specificity but are limited by cost, infrastructure, and turnaround time, particularly in settings with limited resources (Muntean *et al*., 2022). In addition, the continuous emergence of new β-lactamase variants and mobile genetic elements complicates diagnostic standardization and breakpoint interpretation (Wenzler *et al*., 2023). These limitations contribute to delays in appropriate therapy and increase the risk of treatment failure.

Furthermore, the heterogeneity observed in clinical studies, including variations in pathogens, resistance mechanisms, and patient populations, complicates the interpretation of treatment outcomes and limits the broad application of the results (Lodise *et al*., 2022). As a result, current diagnostic and surveillance systems may underestimate the true burden of antimicrobial resistance, reinforcing the need for integrated approaches that combine phenotypic, molecular, and epidemiological data.

CONCLUSIONS AND FUTURE PERSPECTIVES

Antimicrobial resistance in Gram-negative bacteria has become one of the most serious challenges in modern medicine. This review has shown that resistance cannot be traced to a single cause but from the combined action of multiple biological systems working together. These include enzyme-based antibiotic destruction, disturbances with drug entry and increased drug export, and rapid gene exchange between bacteria. Together, these mechanisms allow bacteria to survive even when strong antibiotics are used, making infections harder to treat and control in both hospitals and communities. Understanding these processes as a connected system is important for improving treatment strategies and public health responses.

Key takeaways

- Gram-negative bacteria use β-lactamase enzymes to break down important antibiotics, including carbapenems, making many first-line treatments ineffective.
- Efflux pump systems work to actively remove antibiotics from bacterial cells, lowering drug concentration and reducing treatment success.
- Horizontal gene transfer allows bacteria to rapidly share their resistance genes, which

leads to fast spread across different species and regions.

- These mechanisms often work together in the same bacterial cell and it creates stronger and more complex resistance patterns than any single mechanism alone.
- The combination of enzymatic resistance, efflux activity, and gene transfer explains the high success of multidrug-resistant Gram-negative pathogens in clinical settings.

Unanswered questions

Despite major advances in the field, there are several important questions that are yet to be answered. First, there is the struggle to completely understand the full extent of how different resistance mechanisms interact at the molecular level. Second, it is not fully clear how environmental conditions in hospitals and communities influence the speed of resistance development and spread. Third, more research is needed to understand how resistance and virulence genes co-evolve within the same mobile genetic elements.

Future research directions

Future research should focus on improving rapid diagnostic tools that can detect multiple resistance mechanisms at the same time. This will help clinicians choose effective treatments earlier and reduce treatment failure. Another important direction is the development of new drugs, including improved β-lactamase inhibitors and efflux pump blockers, which can work against highly resistant bacteria. In addition, expanding genomic surveillance using whole-genome sequencing and One Health approaches will help track resistance across humans, animals, and the environment in real time.

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