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Review article

Antimicrobial resistance mechanism among Gram-negative bacteria: A mini review

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Abstract: Antimicrobial resistance (AMR) has emerged as a pressing global health issue, primarily fueled by the inappropriate and excessive use of antimicrobial agents, along with the limited progress in developing new and innovative antibiotics. According to the World Health Organization, AMR ranks among the ten most significant threats to global public health. The cell membranes of Gram-negative bacteria play an essential role in their survival and adaptation to different environments. These membranes not only determine the surface characteristics of the bacteria but also serve as protective and functional barriers. Structurally, Gram-negative bacteria possess dual membranes, with the outer membrane comprising proteins and additional components. In many cases, this outer membrane prevents harmful substances such as antibiotics from penetrating, which poses a major challenge in eliminating Gram-negative bacterial infections using both conventional and newly developed antimicrobial agents.

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Introduction

Antimicrobial resistance (AMR) represents one of the most significant global challenges confronting modern medicine and society, yet it remains among the least recognized issues by both healthcare professionals and the general public (Zeb et al., 2022; Zeshan et al., 2021). The overuse and inappropriate distribution of antibiotics have significantly contributed to the emergence of resistant bacterial strains. In many developing countries, antibiotics can often be obtained over the counter without a prescription, which further accelerates this problem. Consequently, raising awareness and educating both patients and the general public are essential steps in combating this growing threat (Zahra et al., 2021). The rising prevalence of resistant pathogens in hospital settings is often linked to the intense selective pressure created by the frequent use of certain antimicrobial agents in patients. This is especially true for drugs such as extended-spectrum cephalosporins, β-lactam–β-lactamase inhibitor combinations, carbapenems, fluoroquinolones, and aminoglycosides (Zahari et al., 2023). The AMR has not only restricted the available treatment options but has also contributed to higher healthcare costs and increased mortality in both humans and animals. Given the limited pipeline of new antimicrobials to address severe and life-threatening infections, AMR is now recognized as one of the most critical and enduring threats to global health (Yusof et al., 2022).

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Ecological Perspectives

In recent decades, the development and progression of AMR have been the subject of extensive review and analysis (Wada et al., 2024; Wahab et al., 2021). Bacteria generally exist within complex, multi-species communities, where interactions among different microorganisms significantly influence how they respond to antibiotic exposure. These dynamics carry important clinical, ecological, and environmental implications, shaping tolerance levels, driving the selection of resistant strains, and influencing the overall course of resistance evolution. Traditionally, assessments of a pathogen's antibiotic susceptibility are based on pure culture studies conducted under relatively uniform conditions (Tariq et al., 2020). While such data may be sufficient for treating infections caused by a single strain, they often fail to accurately reflect the susceptibility of a pathogen during polymicrobial infections or when it resides within a broader commensal microbial community (Sohail et al., 2023).

Both human medicine and food production depend greatly on the effective use of antibiotics. However, the growing AMR observed in human and animal pathogens poses a critical threat to public health and agricultural productivity. As bacterial populations continue to evolve rapidly, many conventional antibiotics are losing their effectiveness, undermining treatment strategies and food safety alike (Ramzan et al., 2024; Ramzan et al., 2022).

Epidemiological trends in Gram-negative bacteria

One of the major drivers of emerging resistance is poor antibiotic stewardship, which results in the overuse of antimicrobials, inappropriate empiric treatments, and delays in establishing accurate diagnoses. Resistance is particularly pronounced among patients in acute care settings, such as intensive care units, where it contributes to alarmingly high mortality rates ranging from 26% to 80%. To optimize outcomes, antimicrobial therapy should always be guided by the identification of the causative pathogen and subsequent susceptibility testing (Rizvi et al., 2022). In addition, individual patient factors—such as drug allergies and kidney or liver function—must be considered when selecting appropriate treatments. Within the spectrum of hospital-acquired infections (HAIs), drug-resistant Gram-negative bacteria are becoming increasingly common. In the United States, the majority of Gram-negative HAIs are caused by *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli,* and *Acinetobacter baumannii*, all of which are showing alarming levels of resistance to currently available antibiotics (Shunmugam et al., 2025). Modern medicine relies heavily on antibiotics for the safe treatment of bacterial infections, which are central to procedures such as abdominal operations, organ transplants, and cancer care. Without effective antibiotics, these medical interventions would carry unacceptable risks of infection and could not be performed safely (Singh et al., 2024).

Bacteria are likely to continue developing resistance to existing antibacterial drugs, either via novel mutations or by sharing resistance genes with other strains. Across many healthcare settings, multidrug resistant (MDR) pathogens are becoming increasingly prevalent, complicating patient management and driving up both morbidity and medical expenses. Choosing the appropriate drug for each infection is critical to mitigating these challenges (Rasool et al., 2022; Rizvi et al., 2022). Bacterial AMR is a pressing problem associated with high rates of illness and death. Treating multidrug-resistant Gram-positive and Gram-negative bacteria is challenging, and conventional antibiotics may sometimes fail entirely (Rabaan et al., 2022b).

The current lack of effective therapies, limited preventive strategies, and slow development of new antibiotics underscore the need for innovative treatment options and alternative antimicrobial approaches. Biofilms contribute to MDR and complicate infection management. High-risk pathogens such as *Staphylococcus aureus, Clostridium difficile,* and vancomycin-resistant *Enterococci* highlight the importance of stringent infection control, especially in Emergency Department settings (Rabaan et al., 2022b). *Enterobacteriaceae* are key pathogens responsible for severe infections, and resistance to currently available antibiotics among these bacteria is on the rise (Rabaan et al., 2022a).

Mechanisms of antimicrobial resistance in Gram-negative bacteria

CAMPs are positively charged antimicrobial peptides known for their ability to kill or inhibit a broad range of pathogens, such as bacteria, viruses, fungi, and parasites. These peptides form a highly diverse family produced across many life forms—from simple prokaryotes to complex vertebrates—and over 1,200 different types have already been documented (Zahari et al., 2023). Defensins, cathelicidins, and thrombocidins are examples of cationic antimicrobial peptides (CAMPs) that serve as a crucial part of human innate defense, safeguarding epithelial barriers and skin while aiding neutrophils and platelets in fighting infection. Pathogens such as *S. aureus* and *Salmonella enterica* have evolved multiple mechanisms to resist CAMPs. These include decreasing the negative surface charge of the bacterial envelope by modifying anionic components like teichoic acids, phospholipids, and lipid A; pumping CAMPs out through energy-dependent systems; changing the physical properties of their membranes; and neutralizing CAMPs by producing proteases (Rabaan et al., 2022b).

Enzymatic degradation of antibiotics

Many antibiotic molecules possess hydrolytically sensitive bonds, including esters and amides, which are crucial for maintaining their biological effectiveness. Several enzymes have adapted to exploit this weakness by cleaving these bonds, ultimately rendering the antibiotic inactive (Rabaan et al., 2022b; Wright, 2005).

The treatment of aerobic Gram-negative infections frequently relies on aminoglycosides, which are commonly paired with β-lactam agents. In the U.S., gentamicin, tobramycin, and amikacin represent the aminoglycosides most widely used in managing serious infections (Naveed et al., 2022a; Naveed et al., 2022b). Aminoglycoside antibiotics function by binding to the A-site of the 30S ribosomal subunit, which plays a key role in verifying codon–anticodon pairing. This binding impairs the 16S rRNA component, leading to inhibition of protein production. Over time, many bacteria have evolved resistance strategies, including chemical modification of the drug, alterations in ribosomal structure, and decreased uptake across the cell membrane (Mustafai et al., 2023).

Alteration of antibiotic targets

Resistance can emerge rapidly against antibiotics that focus on a single enzyme and lack structural similarity to natural substrates. For instance, with rifampicin resistance, multiple single amino acid changes may substantially weaken the drug–target interaction, producing high levels of resistance with clinical impact (Naveed et al., 2022a; Naveed et al., 2022b). For several important pathogen–antibiotic pairs, resistance most commonly develops through mutations or acquired genes that change the antibiotic's binding site. These altered targets may stem from normal chromosomal housekeeping genes, but they can also emerge via horizontal transfer and genetic recombination, producing novel mosaic gene structures (Hughes and Andersson, 2001). Bacterial type II topoisomerases, including gyrase and topoisomerase IV, serve as the main targets of fluoroquinolone antibiotics. These drugs function by stabilizing DNA breaks created by the enzymes and preventing their essential catalytic activity.

Unfortunately, resistance has emerged due to mutations in these enzyme targets, diminishing fluoroquinolone effectiveness (Parveen et al., 2020). To address this, new drug classes have been developed that still act on type II topoisomerases but bind through different amino acid interactions. Recent discovery efforts have produced innovative antibacterials, and two of them—gepotidacin and zoliflodacin—have successfully completed Phase III clinical trials with promising outcomes (Eltayeb et al., 2023; Hussain et al., 2022).

Efflux pumps

Both bacterial and eukaryotic cells use active efflux mechanisms that significantly influence antibiotic activity. Many types of efflux pump capable of transporting antibiotics have now been characterized. Among eukaryotes, primary active transporters are especially prominent, with six families of the ATP-binding cassette (ABC) superfamily such as P-glycoprotein in the MDR group and the MDR protein (MRP) identified as central

players in antibiotic export (Zahari et al., 2023).

Research findings from clinical and experimental settings suggest that efflux pumps play a dual role facilitating drug removal while also contributing to virulence and adaptive mechanisms that support AMR in the course of infection (Du et al., 2018). The development of efflux pump inhibitors has recently been pursued as a strategy to strengthen the activity of antibiotics subject to active export. This method has been applied to design compounds capable of diminishing the role of efflux pumps in fluoroquinolone resistance. Since many efflux systems share notable structural homology, it is anticipated that one inhibitor could be effective across different bacterial species (Webber and Piddock, 2003).

Reduced membrane permeability

Unlike Gram-positive bacteria (Ejaz et al., 2024; Hussain et al., 2022), which maintain a more rigid wall structure, Gram-negative bacteria exhibit a different organization. Their cell envelope includes an outer membrane positioned above a thin peptidoglycan layer. The region between the outer membrane and the plasma membrane, called the periplasmic space, contains a dense gel-like substance referred to as the periplasm. As it lies above the plasma membrane, the periplasm does not belong to the protoplast, and because it is enclosed by the outer membrane, it is also distinct from the external environment (Absar et al., 2025).

Since most antibiotics function inside bacterial cells, they must first penetrate the protective envelope. For Gram-negative bacteria, the outer membrane represents a major obstacle. Entry can occur through two distinct mechanisms: hydrophobic antibiotics use a lipid-based pathway, whereas hydrophilic ones cross via diffusion porins. Variations in the lipid and protein composition of the outer membrane significantly affect bacterial sensitivity, and resistance frequently develops through alterations to these macromolecules (Absar et al., 2025).

Horizontal gene transfer

In horizontal gene transfer (HGT), DNA either single or double-stranded is exchanged from one cell to another. HGT is a key driver of bacterial genome flexibility and plays a vital role in adaptation and evolution, particularly through the transfer of resistance determinants and virulence-associated genes. The process typically relies on mobile genetic elements (MGEs), such as plasmids and transposons, which carry and disseminate these genes (Liu et al., 2022). Through various mechanisms, HGT allows genes to escape the limitations of vertical transmission. Genetic exchange can occur via plasmid conjugation, bacteriophage transduction, or uptake of extracellular DNA during natural transformation. This capacity adds a critical layer to the progression of infectious diseases, as a single AMR gene can spread to multiple distinct pathogens, driving widespread outbreaks (Lerminiaux and Cameron, 2019).

In Gram-negative bacteria, conjugation, transformation, and transduction are the primary mechanisms through which antibiotic resistance genes (ARGs) are acquired and dispersed. The plasmid-mediated transfer of multiple ARGs is particularly alarming, as it can instantly render sensitive strains multidrug resistant. Bacteriophage-driven transduction, which involves packaging ARG-containing chromosomal DNA, also plays a major role in resistance spread without direct contact between cells. Recently, outer membrane vesicles (OMVs) have been recognized as a novel HGT mechanism that significantly contributes to ARG transfer (Wachino, 2025).

Biofilm formation

Bacterial biofilms consist of aggregated cells encased in an extracellular network of polysaccharides, proteins, nucleic acids, and enzymes, allowing them to adhere firmly and permanently to surfaces (Ahmed et al., 2022e; Ahmed et al., 2019). When biofilm formation occurs alongside β-lactamase activity, the spread of multidrug-resistant Gram-negative bacilli is greatly enhanced. Such biofilms are associated with long-lasting, difficult-to-eradicate infections that often recur, leading to significant illness and death, thereby creating a critical healthcare challenge (Ahmed et al., 2022c; Ahmed et al., 2022d).

The presence of AMR among Gram-negative bacteria in dairy products is worrisome, as they can pass resistance traits to human gut microbes and negatively influence product quality (Amin et al., 2023; Assiry et al., 2023). One of the key survival strategies of these bacteria is biofilm formation, which enables them to persist throughout the food chain (Afzal et al., 2025; Afzal et al., 2023; Ahmed et al., 2022b). For this reason, identifying Gram-negative bacteria at multiple points during the manufacturing process and evaluating their ability to produce biofilms is of great importance (Woo et al., 2023).

Clinical significance of resistance in Gram-negative bacteria

Dealing with AMR in Gram-negative bacilli (GNB) is a constant clinical obstacle in ICU. Reports from across the world highlight escalating resistance rates, with predictions of even greater challenges ahead (Ahmed et al., 2025; Ahmed et al., 2020).

The microbial landscape of every ICU differs, shaped by factors such as antibiotic stewardship policies, the type of patients admitted, and the occurrence of sporadic outbreaks (Ruppé et al., 2015). The issue of AMR is particularly troubling for healthcare providers, as it leaves only a few viable treatment options and sometimes none at all. In *Enterobacteriaceae* species, including *E. coli, Klebsiella*, and *Enterobacter*, resistance to first- through fourth-generation cephalosporins is largely mediated by ESBLs. As AMR continues to escalate, it not only narrows therapeutic choices but also increases the financial burden of treatment and elevates death rates across human and animal populations (Abusalah et al., 2024; Afzal et al., 2025).

Detection and surveillance of resistance

The recognition of carbapenemase-producing (CP) bacteria in clinical labs is essential for guiding therapy and applying infection prevention measures. Yet, detection remains problematic since it cannot rely solely on resistance patterns, and current test methodologies have not been fully standardized (Ahmed et al., 2020; Ahmed et al., 2022a).

Worldwide, critically drug-resistant GNB are responsible for considerable rates of morbidity and mortality. Among the leading pathogens observed in clinical practice are *E. coli, Klebsiella pneumoniae*, the *Acinetobacter baumannii* complex (ABC), and *P. aeruginosa* (Yungyuen et al., 2021). Continuous improvement in molecular diagnostics is crucial for effective surveillance of bacterial infections. Despite progress in antibiotic susceptibility testing, several limitations pe Recognizing AMR markers at an early stage, particularly those tied to cephalosporin and carbapenem resistance, is essential for effective management of bloodstream infections triggered by Gram-negative bacteria such as difficulties in applying these methods directly to clinical samples, reliance on costly instruments, and the requirement for specialized extraction methods to identify resistance biomarkers. Fast and accurate detection of serious infections is key to starting timely therapy, reducing inappropriate antibiotic prescriptions, and decreasing morbidity and overall treatment costs (Ahmed et al., 2022c; Gerace et al., 2022).

Misuse of antibiotics

The AMR, while a natural evolutionary process, has been significantly hastened by decades of inappropriate and excessive antibiotic use in humans and animals. Epidemiological evidence clearly demonstrates that the overuse of antibiotics directly contributes to AMR. Yet, despite numerous global health campaigns, the misuse of these drugs continues unchecked in many parts of the world, raising fears that the problem is reaching an irreversible stage (Zeb et al., 2022).

Studies reveal that large sections of the global population, particularly those with limited education, hold misconceptions—for instance, that antibiotics are effective against viral infections such as influenza or the common cold. In developing countries with inadequate diagnostic infrastructure, antibiotics are often prescribed excessively, even when not clinically indicated (Rabaan et al., 2022b).

The ease of purchasing antibiotics over the counter for both human and veterinary use also accelerates the spread of resistance. This problem is compounded by the lack of effective antibiotic stewardship policies and treatment protocols in many regions. Overprescription by healthcare workers, pharmacists, and veterinarians remains common, especially in underdeveloped nations. Poor-quality or counterfeit antibiotics

in circulation further undermine treatment outcomes. Additionally, inappropriate practices such as prescribing unnecessarily long courses or incorrect dosages of antibiotics contribute heavily to resistance. In some instances, unethical motivations including financial incentives from pharmaceutical companies or pressure to meet patient expectations lead physicians to prescribe antibiotics without justification (Salam et al., 2023).

Strategies to overcome resistance

Historically, natural products have served as valuable treatments for many diseases for instance, quinine from the cinchona tree against malaria and penicillin against infectious illnesses. Following Fleming's landmark discovery of penicillin in 1929, countless antibacterial drugs have been created, dramatically reducing mortality and enhancing public health worldwide. Yet, the growing problem of AMR has spurred major international efforts to discover new agents or improve existing ones. Resistance arises when bacteria evade antibiotics by degrading them, exporting them through efflux pumps, or altering their cell structures to prevent drug binding. Recently, scientists have renewed their focus on natural product-based approaches, viewing them as a rich source of potential new antibiotics. Here, we discuss novel therapeutic strategies emerging from research and development initiatives to address resistant Gram-negative pathogens (Breijyeh et al., 2020).

Initially, antibiotics were designed to combat Gram-positive cocci, but this approach soon resulted in the selection of Gram-negative strains resistant to β-lactams. The rise of diverse β-lactamase enzymes in enteric bacteria prompted researchers to prioritize alternative classes of drugs, such as aminoglycosides and tetracyclines. This was later followed by efforts to optimize fluoroquinolone compounds, offering an effective way to bypass β-lactam resistance in Gram-negative organisms (Bush, 2016). Antimicrobial therapy entered its modern phase in the 19th century with the introduction of chemically synthesized organic agents against syphilis and trypanosome-related diseases. With the rapid escalation of AMR, certain pathogens have now been labeled as top priorities for research and treatment innovation. Effective control measures are urgently needed, particularly the development of new antibiotic options. In addition, vaccines can contribute significantly by limiting unnecessary antibiotic use, decreasing the emergence of resistant variants, and directly acting against resistant microbes (Rosini et al., 2020). The role of vaccines in combating resistance lies in their ability to lessen the dependence on antimicrobials and reduce infection rates.

By minimizing the range of pathogens linked to specific clinical conditions, vaccines make it possible to employ more targeted, narrow-spectrum antibiotics in empirical treatment strategies (Lipsitch and Siber, 2016). To combat AMR worldwide, it is essential to rely on both vaccines and antibacterial measures. Each of these approaches is supported by strong scientific evidence that equips them to meet this pressing need (Baker et al., 2018). Globally, pneumonia remains the top infectious killer of children, making it imperative to consider effective interventions. Antibiotic therapy is highly effective for bacterial pneumonia, and access to these medications can be life-saving. It is estimated that providing antibiotics universally to children under the age of five could avert roughly 445,000 deaths per year in 101 countries. However, misuse or overuse of antibiotics risks promoting AMR, representing a serious threat to public health (Mullins et al., 2023).

As one of the major global health threats of our time, AMR demands urgent attention. This review systematically addresses the impact of resistance among critical pathogens and assesses existing therapies along with emerging treatments in preclinical and clinical stages, giving clinicians an updated overview of available and prospective options (Gajic et al., 2025). Bacterial resistance remains a pressing issue worldwide. Recent genetic exchanges from harmless, saprophytic species to pathogenic organisms like *S. pneumoniae* and *N. meningitidis* have modified penicillin targets, fueling rapid increases in penicillin resistance. Among Enterobacteriaceae, consecutive minor mutations have generated new β-lactamases with broadening activity, now compromising almost all β-lactam antibiotics. Resistance is appearing both in hospital environments and increasingly among community bacteria. While antibiotic overuse, misuse, and abuse are probably major contributors, proving direct causation is difficult (Rabaan et al., 2022a).

Certain countries and hospitals display unusual resistance patterns that correspond to specific antibiotic practices. Addressing this challenge requires coordinated efforts. On a global scale, establishing clear antibiotic policies, implementing comprehensive educational programs on antibiotic use and cross-infection

control, and monitoring pharmaceutical marketing strategies are critical. Locally, institutions should adopt customized guidelines, maintain updated formularies guided by scientific evidence, employ molecular tools to track subtle epidemic shifts and emerging resistance genes, and ensure that physicians receive regular updates on resistance trends to inform prevention and treatment strategies (Absar et al., 2025).

Conclusion

The global spread of multidrug-resistant organisms in healthcare settings poses a serious threat, especially to patients in intensive care units who face heightened exposure risks. Gram-negative infections resistant to many or all antibiotics are increasingly difficult to treat. Ensuring the continued efficacy of antibiotics depends on their judicious and effective use by clinicians. Available treatment options are limited, particularly due to the emergence of carbapenem-resistant strains.

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