Carbapenem Resistance Mechanisms in *Enterobacteriaceae*: Molecular Mechanisms, Epidemiology, and Clinical Implications

Introduction

Carbapenems have become essential in managing infections caused by multidrug-resistant *Enterobacteriaceae*, particularly in severe hospital-acquired cases where other antibiotics fail. However, the emergence and rapid spread of carbapenem-resistant *Enterobacteriaceae (CRE)* have severely reduced treatment efficacy and raised concern across the globe. Between 2012 and 2017, surveillance data indicated a sharp rise in carbapenem resistance rates among common pathogenic *Enterobacteriaceae* such as *K. pneumoniae* and *E. coli*, with resistance rates in some regions reaching as high as 50% (Kazmierczak, 2021; Tian, 2020). These resistant strains not only prolong hospital stays and increase treatment costs but are also associated with high mortality rates in immunocompromised and critically ill patients (Alizadeh, 2020).

The global spread of CRE is driven by the combination of healthcare-associated and community-acquired strains. In South Asia, for example, the overuse of carbapenems in hospitals has contributed to a high prevalence of CRE, including strains carrying metallo-beta-lactamase genes like New Delhi Metallo-beta-lactamase (NDM), which complicates infection control efforts (Farzana , 2022). Similarly, recent findings indicate that the NDM enzyme has spread to Switzerland, highlighting the mobility of carbapenemase-producing strains and the risk of international transmission (Findlay, 2021) Figure 1, from Kazmierczak et al. (2021), shows resistance patterns to meropenem, a common carbapenem drug, on a global scale. The percentage of isolates showing meropenem resistance was low, about 3.3% globally, but that is 3.3% of 81,781 isolates collected from 2021-2017. As of 2022, global resistance had risen up to about 8% with the maximum being 12.9% of isolates from Latin America and the lowest being 1.1% of isolates from North America (Wise, 2024).

This paper explores the primary molecular mechanisms that confer carbapenem resistance to members of the family *Enterobacteriaceae*, including enzymatic degradation by carbapenemases, plasmid-mediated gene transfer, outer membrane porin modifications, and efflux pump activation. The paper also considers the clinical implications of CRE, emphasizing the treatment challenges posed by these resistant strains and the need for innovative therapeutic and containment strategies.

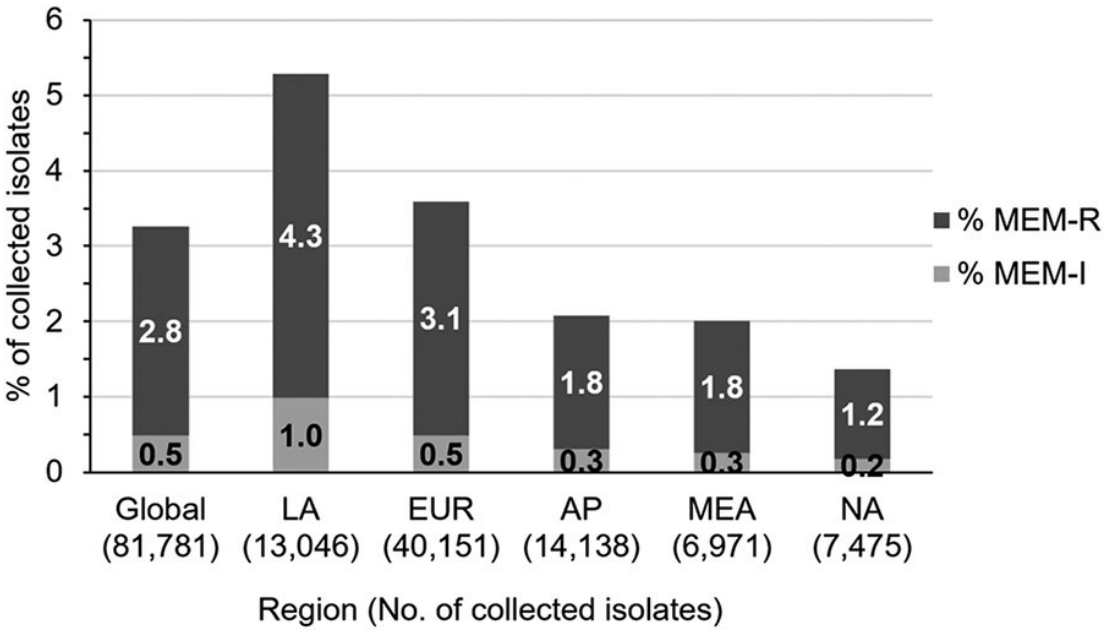

Figure 1: In an epidemiological study by Kazmierczak et al. (2021) 81,781 CRE isolates were collected from various regions around the world. MEM-R denotes meropenem resistance and MEM-I denotes an intermediate meropenem effect on isolates. The region abbreviations are LA for Latin America, EUR for Europe, AP for Asia and South Pacific, MEA for the Middle East and Africa, and NA for North America.

Discussion

Plasmid-Mediated Resistance and Gene Transfer

One major mechanism of carbapenem resistance in Enterobacteriaceae is plasmid-mediated gene transfer. Plasmids can carry multiple resistance genes, and horizontal transfer enables rapid dissemination of these genes between bacteria. Additionally, plasmid multimerization, where multiple copies of carbapenemase genes aggregate within a single plasmid, has been observed to amplify resistance levels (Abe, 2021). This phenomenon is particularly concerning as it permits the spread of carbapenem resistance across both hospitals and communities, with *K. pneumoniae* and other *Enterobacteriaceae* strains acting as primary vectors for gene transfer (Ruekit, 2021). It is feasible for other resistance genes to be present on plasmids, thus this mechanism may be responsible for enhancing the mechanisms explored later in the paper.

To investigate plasmid-mediated resistance, researchers have employed whole-genome sequencing and plasmid analysis. These methods allow for the identification of plasmids and the specific resistance genes they carry, providing a detailed view of the genetic elements responsible for resistance spread (Farzana, 2022). In regions like South Asia, where CRE prevalence is high, plasmid-mediated resistance plays a pivotal role in driving local and regional outbreaks, highlighting the need for rigorous antimicrobial stewardship and targeted containment strategies (Kazmierczak, 2021).

Enzymatic Degradation by Carbapenemases

The most effective mechanism of carbapenem resistance involves the production of carbapenemases—enzymes that hydrolyze carbapenem antibiotics, rendering them ineffective. Among the most common carbapenemases are NDM and OXA-48, both of which break down the beta-lactam ring structure of carbapenems and neutralize their antibacterial properties (Aurilio, 2022). These enzymes are particularly prevalent in strains of *K. pneumoniae* and *E. coli*, which frequently have multiple resistance genes and are significant treatment challenges in clinical settings (Shi, 2022).

Studies examining carbapenemase production utilize PCR amplification and multilocus sequence typing to identify and categorize carbapenemase genes within clinical isolates (Hadjadj, 2021; Kazmierczak, 2021; Tian, 2020). Through these techniques, genes like bla_{NDM} and bla_{OXA-48} are detected, enabling tracking of the prevalence and spread of these enzymes across various regions (Kazmierczak , 2021). The rapid identification of genes like these are essential for informing treatment options, as it allows healthcare providers to assess resistance patterns within hospital settings. Given the high mutation rate and adaptive capacity of carbapenemase genes, ongoing surveillance is crucial to predict and mitigate future outbreaks (Kazmierczak, 2021).

Outer Membrane Porin Modification

Outer membrane porin modifications represent another mechanism by which *Enterobacteriaceae* evade the effect of carbapenems. Porins are channels that allow small molecules, including antibiotics, to permeate bacterial cells. However, genetic mutations in porin channels reduce carbapenem influx, lowering its intracellular concentration and thereby diminishing its bactericidal effect (Little, 2012; Ye, 2024). This resistance mechanism frequently co-occurs with carbapenemase production in *E. coli* and *K. pneumoniae* strains, resulting in multi-layered resistance that complicates treatment (Alizadeh, 2020).

Research into porin modifications has utilized transcriptomic analysis to examine changes in porin gene expression. This approach allows researchers to detect mutations that alter porin channel structure and function, providing insight into how these modifications impact antibiotic permeability (Qiu, 2024). Porin modifications thus represent a critical factor in CRE resistance, and the ongoing development of diagnostic tools to detect such changes is essential for effective patient management.

Efflux Pumps Reduce Intracellular Antibiotic Concentration

Efflux pumps are another resistance mechanism which enables CRE to actively expel antibiotics, reducing their intracellular concentration. These pumps are commonly found in multidrug-resistant strains of *K. pneumoniae* and other CRE, and their presence further complicates treatment by diminishing antibiotic efficacy (Alizadeh, 2020). The most notorious pump system in CRE is the AcrAB-TolC multidrug resistance tripartite pump, which acts on a wide variety of substrates (Pérez, 2007; Guérin, 2016). The concurrent activity of efflux pumps with other

resistance mechanisms, such as carbapenemase production and porin alterations, creates a formidable barrier to antibiotic penetration and retention (Sureka, 2021).

The role of efflux pumps in resistance has been studied using molecular assays and efflux pump inhibitors, which help to isolate and measure pump functionality (Alizadeh, 2020). By inhibiting efflux pump activity, one can determine the extent to which these pumps contribute to resistance, distinguishing efflux-mediated resistance from other mechanisms. This approach is important for identifying potential drug targets, as it highlights the importance of efflux pumps in multidrug resistance and informs future therapeutic development.

Carbapenem Resistance Mechanisms

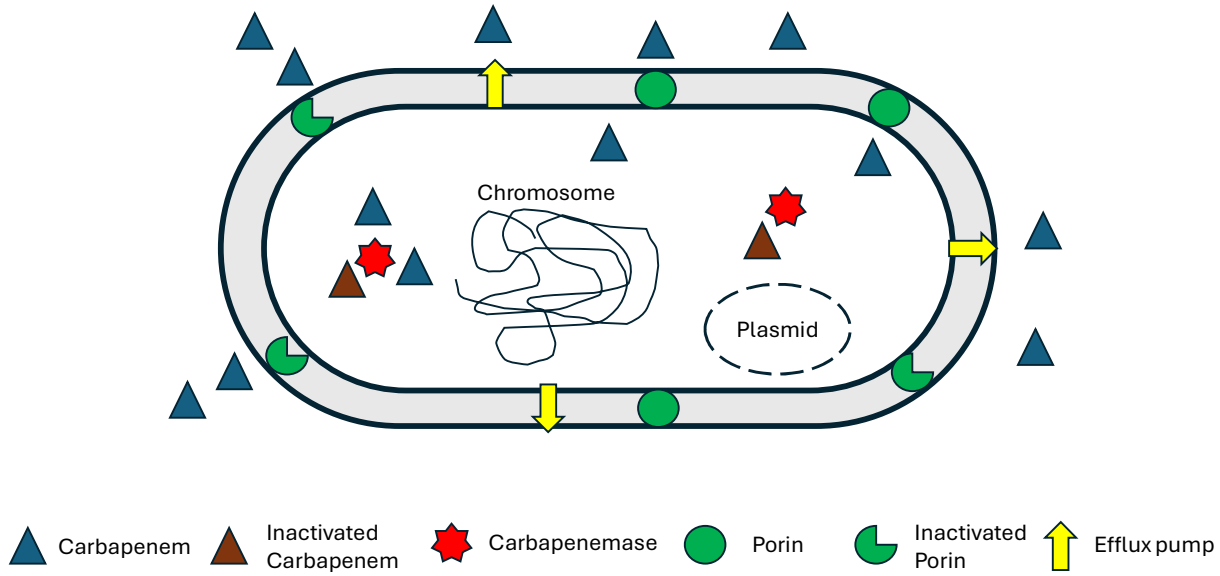

Figure 2: This figure summarizes the resistance mechanisms described in the prior sections. Efflux pumps move carbapenem from within the cell to the exterior, plasmids encoding resistance genes may be transferred between bacteria, carbapenemases hydrolyze carbapenem and render it inactive, and porins may be modified or under-expressed to reduce carbapenem influx.

Environmental Influences on Resistance Mechanisms

Recent studies indicate that environmental factors, such as herbicide exposure, may indirectly contribute to resistance in *Enterobacteriaceae*. Zerrouki et al. (2024) demonstrated that glyphosate-based herbicides, when combined with antibiotics, can induce phenotypic changes in Gram-negative bacteria, suggesting a possible link between agricultural practices and clinical resistance. This connection between environmental factors and antibiotic resistance shows the complexity of resistance development and the need for interdisciplinary research to address these influences.

To explore this phenomenon, researchers combined glyphosate with antibiotics in experimental assays, observing that environmental exposures could enhance resistance traits (Zerrouki, 2024). The findings suggest that non-antibiotic agents, when present in bacterial

environments, may create a selective pressure favoring bacteria with resistance mechanisms, further complicating resistance containment. This area of research calls for closer examination of how environmental conditions influence bacterial adaptation and resistance, but it is an interesting avenue which has not seen much investment.

Clinical Implications

Challenges in Treatment and Control

The treatment of CRE infections is challenging due to the limited antibiotic options available for resistant strains. Many CRE strains resist multiple drug classes, including beta-lactams, aminoglycosides, and fluoroquinolones, requiring last-resort drugs such as colistin, which carry risks of nephrotoxicity (Stewart, 2018). OXA-48-producing strains, for example, resist almost all beta-lactam antibiotics, leaving clinicians with few effective treatments (Findlay, 2021). Consequently, patients infected with CRE strains are at increased risk of treatment failure, prolonged illness, and mortality (Shi, 2022).

Implications for Antimicrobial Stewardship and Infection Control

Controlling the spread of CRE requires stringent antimicrobial stewardship and infection control measures, particularly in healthcare settings. Surveillance programs that track carbapenemase gene prevalence are essential for identifying resistance hotspots and implementing targeted interventions (Kazmierczak, 2021). Infection control protocols, including patient isolation and exceptional sanitation, are crucial in preventing CRE transmission, especially in hospitals where resistant strains can rapidly spread (Olowo-Okere , 2020).

Effective stewardship programs also involve restricting the use of carbapenems as a first line of treatment, thereby reducing selective pressure on bacterial populations and minimizing resistance spread. Given the role of plasmid-mediated gene transfer in CRE dissemination, controlling antibiotic use is vital to contain resistance and prevent widespread outbreaks (Kazmierczak, 2021).

Future Directions in Therapeutic Research

Due to the limited efficacy of current treatments, novel therapeutics and adjunctive therapies are urgently needed. Future research should focus on developing agents that target specific resistance mechanisms, such as efflux pump inhibitors or compounds that deactivate carbapenemase enzymes (Shi , 2022). Another promising area of study is the inhibition of plasmid-mediated gene transfer, which could prevent the horizontal spread of resistance genes across species (Abe , 2021). One example of neutralization of resistance was shown by Hao using a CRISPR-Cas9 system to mitigate resistance using NDM and OXA-48 carbapenemases (Hao, 2020). Their hurdle is finding a method of therapeutic delivery. Additionally, Zerrouki (2024) suggest the need for more research into environmental impacts on resistance, as understanding these factors may help mitigate resistance in non-clinical settings.

Conclusion

Carbapenem resistance in *Enterobacteriaceae* is a complex phenomenon driven by multiple mechanisms, including carbapenemase production, plasmid-mediated gene transfer, porin modifications, and efflux pump activity. These mechanisms not only challenge treatment options but also contribute to the rapid global spread of resistance. The clinical impact of CRE is profound, particularly in settings with limited resources, underscoring the importance of robust antimicrobial stewardship and infection control. Moving forward, research into new therapeutics, combined with broader ecological approaches, is essential to combat CRE and preserve the efficacy of last-resort antibiotics.

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