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Review Article

The Role of Plasmids in Antibiotic-resistance: *Klebsiella pneumoniae* as a Model

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ABSTRACT

Plasmids are extrachromosomal DNA molecules that replicate within bacterial cells. These mobile genetic elements play a critical role in bacterial evolution, particularly in the adaptation to environmental pressures such as antibiotic exposure. Plasmids can carry genes that confer various survival advantages, including antibiotic resistance genes (ARGs). Through horizontal gene transfer (HGT), plasmids enable rapid genetic exchange between bacterial populations, facilitating the spread of resistance traits across species and genera. As a result, plasmids are central players in the emergence and dissemination of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens, including *Klebsiella pneumoniae* (*K. pneumoniae*), a leading cause of hospital-associated infections.

In this review, we focus on the role of plasmids in the evolution of antibiotic resistance in *K. pneumoniae*, with a particular emphasis on the molecular mechanisms underlying plasmid-mediated resistance and their contribution to the global crisis of antimicrobial resistance.

KEYWORDS

Plasmids, K. pneumoniae infection, Antibiotic-resistance genes.

INTRODUCTION

Antibiotic resistance is one of the most pressing global health challenges of this century. The overuse and misuse of antibiotics in both healthcare and agriculture have contributed to the alarming increase of multidrug-resistant (MDR) pathogens [1]. Among these, *Enterobacteriaceae,* such as *K. pneumoniae,* represent a serious threat to the global public health [2].

K. pneumoniae is an opportunistic pathogen responsible for a wide range of infections, particularly in hospitalized, immunocompromised, and critically ill patients. It is responsible for pneumonia, urinary tract infections, sepsis, and wound infections [3]. Despite *K. pneumoniae* is a common inhabitant of the human gastrointestinal microbiota [2], it has become the main cause of both hospital-acquired and community-acquired infections. The developed capacity of the bacterium to produce enzymes that degrade the β-lactam structure of antibiotics has contributed to worsen this concern, making ineffective the use of carbapenems - the last-line antibiotics used to treat serious infections - thus favoring the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) [4].

One of the primary causative mechanisms of the spread of antibiotic resistance in *K. pneumoniae* and other bacteria is the horizontal transfer of resistance genes via mobile genetic elements, especially plasmids.

Plasmids are extrachromosomal, self-replicating DNA molecules that can exist in multiple copies within a bacterial cell [5]. These mobile elements can carry a variety of genes which can elicit important roles in the bacterial evolution by transferring beneficial traits within and between bacteria species, thus contributing positively to bacterial evolution [6]. These genes can confer antibiotic resistance, as well as virulence factors that enhance bacterial survival and pathogenicity [7]. Therefore, plasmids play a crucial role in bacterial adaptation to selective pressures, particularly antibiotic treatment, by facilitating the exchange of genetic material between different bacterial species. This ability to transfer genes, including antibiotic resistance genes (ARGs), via conjugation, transduction, and transformation makes plasmids major contributors to the rapid dissemination of resistance across bacterial populations [8].

In the case of *K. pneumoniae*, plasmids participate to the spread of resistance to multiple antibiotic classes, including β-lactams, aminoglycosides, fluoroquinolones, and polymyxins [4]. Notably, *K. pneumoniae* often harbors large, complex plasmids that can carry multiple resistance

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genes, often in clustered arrangements, which increases the fitness and survival of the bacterial population under antibiotic pressure. Furthermore, plasmids are highly mutable, enabling the rapid evolution of new resistance traits. For example, resistance to β-lactams is frequently conferred by the presence of plasmid-encoded β-lactamases, including extended-spectrum β-lactamases (ESBLs) and carbapenemases [9]. Plasmids that carry these resistance genes have been shown to enhance the level of resistance due to their multicopy nature, which increases gene dosage and the expression of resistance traits.

This review focuses on the role of plasmids in the evolution of antibiotic resistance in *K. pneumoniae*, highlighting the contribution of plasmids to the development and spread of resistance in this clinically significant pathogen. We explore the mechanisms underlying plasmid-mediated resistance, the genetic diversity of plasmids, and the evolutionary implications of plasmid transfer in the context of antibiotic resistance. Understanding the role of plasmids in *K. pneumoniae* resistance is essential for developing more effective strategies to counteract the growing global health threat of antimicrobial resistance.

PLASMIDS

The Biology of Plasmids

Plasmids are extrachromosomal, typically circular DNA molecules that replicate independently of the bacterial chromosome and are often present in multiple copies per cell. In some instances, they may also form plasmid islands clusters of plasmid-related genetic elements integrated into the host genome [10]. Plasmids are generally categorized based on their plasmid copy number (PCN) into high-copy-number plasmids (HCPs) and low-copy-number plasmids (LCPs). HCPs, which can exceed ten copies per cell, rely on their abundance to ensure inheritance during cell division, reducing the chance of loss through segregation. In contrast, LCPs employ tightly regulated partitioning systems to maintain stability at lower copy numbers [11].

A key characteristic of plasmids is their ability to adjust copy number in response to growth conditions or environmental stressors such as antibiotic exposure. While average PCN tends to remain stable across a population, individual cells often show substantial variability in plasmid number [12]. This heterogeneity is biologically significant, as cells with higher plasmid loads may express beneficial traits more robustly. Mutations in replication control elements can further amplify PCN and have been documented in both laboratory and clinical contexts [13]. Notably, some bacteria such as *Aureimonas spp*. carry plasmid-encoded ribosomal RNA (rrn) operons in high copy numbers (18–34 copies per cell), which allows for rapid adaptation to fluctuating environments through enhanced protein synthesis [14].

Plasmids differ fundamentally from chromosomal DNA in their evolutionary dynamics. Their genes often escape genetic dominance or heteroplasmy and experience higher rates of mutation and recombination [15]. These differences allow plasmid-encoded genes to evolve more rapidly, particularly under strong selection pressures. For example, in experimental settings, resistance-conferring mutations in the *blaTEM-1* β-lactamase gene arose readily when plasmid-encoded, but were absent when the gene was chromosomal, even under high mutation supply conditions [16]. This phenomenon is attributed to two main factors: the larger mutational target provided by multicopy plasmids and the amplified expression of beneficial mutations due to increased gene dosage. Another important mechanism enhancing gene dosage is tandem gene duplication, which has been observed particularly in the context of plasmid-encoded resistance genes. These duplications, often found in hetero-resistant populations, further elevate resistance levels and increase survival under antibiotic stress [17]. The coaction of gene amplification and mutational plasticity allows plasmids to act as rapid-response elements in bacterial adaptation and evolution.

Plasmids and Horizontal Gene Transfer

Plasmids play a critical role in microbial ecology due to their ability to mediate horizontal gene transfer (HGT). HGT enables the transfer of genetic material between different bacterial strains, species or genera, bypassing the slower vertical inheritance associated with chromosomal genes. The primary mechanism of plasmid-mediated HGT is conjugation, which requires direct cell-to-cell contact and facilitates the spread of plasmids carrying adaptive traits such as virulence factors or antibiotic resistance genes (ARGs). However, plasmids can also be mobilized via transduction, mediated by bacteriophages, or transformation, involving the uptake of free plasmid DNA from the environment [7] (Figure 1). Importantly, plasmid variability occurs within genetically identical (clonal) populations. Studies have demonstrated significant variation in plasmid number and configuration across individual cells, contributing to phenotypic heterogeneity. This has been documented not only in well-studied species like *Escherichia coli* and *Klebsiella pneumoniae*, but also in phylogenetically distant bacteria, such as *Yersinia spp*. and *Agrobacterium tumefaciens*, in which plasmids respond to host signals to modulate their behavior [18-20]. These findings highlight the ecological flexibility and evolutionary resilience conferred by plasmids.

Plasmids as Vectors of Antibiotic Resistance

The rise of antibiotic resistance is one of the most urgent global health threats, driven by both chromosomal mutations and the acquisition of ARGs via mobile genetic elements. Among these, plasmids play a particularly prominent role due to their ability to shuttle ARGs across diverse bacterial hosts. Their mobility, stability, and gene content make them efficient vehicles for resistance dissemination in both community and healthcare settings.

The significance of plasmids in antimicrobial resistance is especially clear in the case of β-lactam antibiotics, where β-lactamase enzymes responsible for hydrolyzing the β-lactam ring are frequently encoded on plasmids. Not only do these plasmid-encoded enzymes confer resistance more effectively than their chromosomal counterparts (likely due to higher gene dosage), but plasmids often carry multiple resistance genes arranged in operons or clusters, enabling multidrug resistance [21]. This gene co-localization facilitates coordinated expression and efficient transmission of complex resistance profiles, accelerating the evolution of MDR or XDR bacterial strains.

KLEBSIELLA PNEUMONIAE INFECTION

The Role of Plasmids in *Klebsiella pneumoniae*

Klebsiella pneumoniae is one of the most prominent opportunistic pathogens responsible for approximately one-third of all Gram-negative bacterial infections worldwide. Although it is a natural inhabitant of the gastrointestinal microbiota of humans and animals, it is capable of causing a wide range of severe extraintestinal infections, including urinary tract infections, cystitis, septicemia, endocarditis, and pneumonia [22]. In addition to nosocomial infections, *K. pneumoniae* is increasingly implicated in community-onset conditions such as necrotizing pneumonia, pyogenic liver abscesses, and endogenous endophthalmitis, which were previously

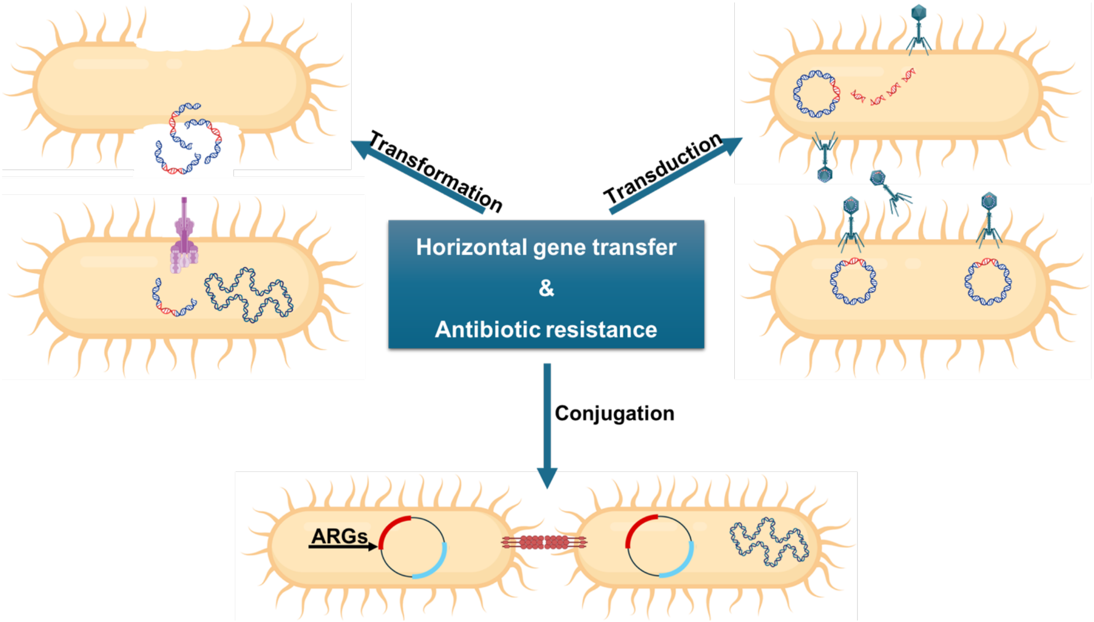

Figure 1: This figure synthesizes three key mechanisms of horizontal gene transfer (HGT) driving the dissemination of antibiotic resistance genes (ARGs). **Transformation:** A bacterial cell internalizes extracellular DNA fragments from its environment. **Transduction:** A bacteriophage transfers plasmid-derived DNA into an acceptor bacterium. This mechanism highlights phage-mediated shuttling of ARGs across bacterial species, contributing to resistance spread in clinical and environmental settings. **Conjugation:** Two bacteria connected by a conjugation pilus exchange a multidrug-resistant plasmid.

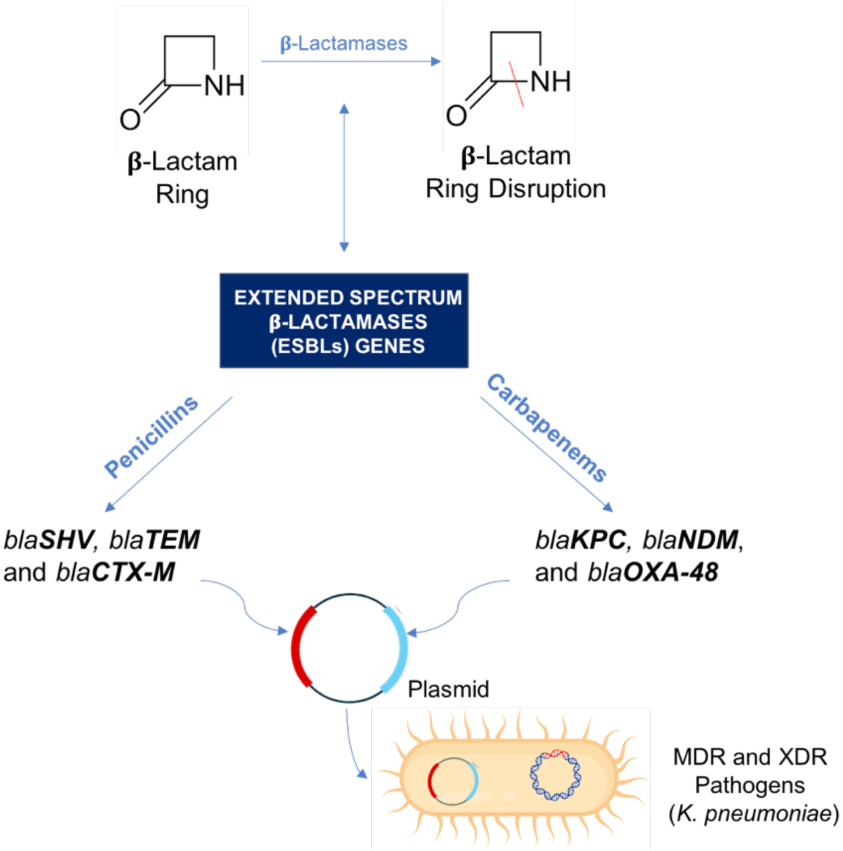

Figure 2: The figure emphasizes how Extended Spectrum β-Lactamases (ESBL)-producing *K. pneumoniae* evades β-lactam antibiotics by enzymatically inactivating their core β-lactam ring structure. β-Lactamases, depicted as hydrolyzing enzymes, target the amide bond within the β-lactam ring of antibiotics such as penicillins (e.g., ampicillin) and carbapenems (e.g., meropenem), rendering these drugs ineffective. ESBL genes (*blaCTX-M, blaTEM, blaSHV*), illustrated as genetic determinants, encode broad-spectrum β-lactamases that confer resistance to penicillins, cephalosporins, and monobactams. These genes, often plasmid-borne, facilitate rapid resistance dissemination across bacterial populations. Carbapenem resistance, a critical clinical concern, emerges when carbapenemase genes (e.g., *blaKPC, blaNDM*) are co-acquired via mobile genetic elements, compounding multidrug resistance.

considered rare but are now recognized globally as emerging clinical threats.

A critical factor in the clinical success of *K. pneumoniae* is its exceptional ability to acquire and disseminate antibiotic resistance genes (ARGs), largely through plasmids. These extrachromosomal elements serve as efficient vehicles for horizontal gene transfer, enabling the rapid acquisition of resistance to multiple antibiotic classes. The most concerning resistance traits in *K. pneumoniae* include genes encoding extended-spectrum β-lactamases (ESBLs), such as *blaSHV, blaTEM*, and *blaCTX-M*; carbapenemases like *blaKPC, blaNDM*, and *blaOXA-48* (Figure 2); and determinants of resistance to aminoglycosides (e.g., *aac(3)-I*) and quinolones (e.g., *qnr* genes). These ARGs are frequently located on large, conjugative plasmids capable of mobilizing additional resistance and virulence factors, thereby enhancing the bacterium's pathogenic potential and adaptability.

The widespread emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *K. pneumoniae* is underscored by surveillance data from the European Antimicrobial Resistance Surveillance Network (EARS-Net). These reports document a consistent increase in resistance rates, particularly in Eastern and Southern Europe and Mediterranean countries, where non-susceptibility to third generation cephalosporins, fluoroquinolones, and aminoglycosides exceeds 50–60%. During this period, carbapenem-resistant *K. pneumoniae* (CRKP) a pathogen of critical concern also emerged, reaching resistance rates as high as 60% in countries like Romania, Italy, and Greece [2,23]. Compared to *Escherichia coli*, *K. pneumoniae* exhibits significantly higher rates of resistance to all major antibiotic classes, including last-resort agents such as carbapenems and polymyxins.

Evolutionary Implications of Plasmid-Mediated Resistance

Plasmids contribute to the evolutionary success of antibiotic-resistant bacteria by promoting genetic diversity within bacterial populations [24]. Due to their high mutation rates and the ability to acquire novel genetic material, plasmids enable bacteria to rapidly adapt to changing environments. The evolution of β-lactam resistance in *K. pneumoniae* provides a prime example of this process. Mutations in the *blaTEM* gene, for example, can lead to the production of enzymes with expanded substrate specificity, thereby conferring resistance to a broader range of β-lactam antibiotics [16]. Furthermore, the interaction between plasmid copy number (PCN) and gene dosage plays a crucial role in the level of resistance conferred by plasmid-borne resistance genes. Increased plasmid copy number often correlates with higher resistance levels, as seen with plasmid-borne β-lactamase genes. Moreover, tandem gene duplications frequently observed in plasmid-encoded ARGs can synergistically increase gene dosage, leading to hetero-resistance and high-level antibiotic tolerance in subpopulations of bacteria [17]. These mechanisms enhance the evolutionary potential of resistance genes, particularly under sustained antibiotic exposure.

K. pneumoniae's resistome, defined as the full complement of ARGs, reflects decades of plasmid-mediated adaptation. Resistance to penicillin was first identified in the 1960s with the discovery of *blaSHV-1* and *blaTEM-1*. In the 1980s, the ESBL variant *blaSHV-2* was isolated in Germany, followed by *blaTEM-3* in France [25], illustrating the historical progression and international dissemination of resistance determinants. These early findings set the stage for the ongoing evolution and global spread of diverse and increasingly potent resistance genes harbored on plasmids.

Global Dissemination of MDR *Klebsiella pneumoniae*

The rise of MDR and XDR *K. pneumoniae* is a global public health crisis driven by the interplay of clonal expansion, plasmid dissemination, and the mobilization of ARGs by other mobile genetic elements such as transposons. High-risk clones, particularly those carrying *blaKPC* and other carbapenemase genes, have been implicated in hospital epidemics worldwide [26]. These clones are often equipped with highly successful multi-resistant plasmids and transposons, allowing them to persist in healthcare environments. Interestingly, the spread of resistance is no longer confined to hospitals. Community-associated MDR *K. pneumoniae* infections are being reported with increasing frequency, indicating that plasmid-mediated resistance is increasing into the general population. This broad dissemination is facilitated by plasmid transfer across species and between commensal and pathogenic strains, enabling ARGs to move through diverse ecological niches. Collectively, these trends underscore the need for integrated surveillance, molecular epidemiology, and alternative strategies aimed at controlling plasmid-mediated resistance in *K. pneumoniae*.

CONCLUSION

In conclusion, plasmids play an essential role in the evolution of antibiotic resistance in *K. pneumoniae*, facilitating the spread of resistance genes within and between bacterial populations. The ability of plasmids to replicate independently, carry multiple resistance genes, and transfer these genes horizontally makes them key drivers of the global antimicrobial resistance crisis. Understanding the molecular mechanisms governing plasmid dynamics, gene transfer, and the evolution of resistance is crucial for developing strategies to counteract the growing threat of multidrug-resistant pathogens.

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