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MOLECULAR CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES IN MULTIDRUG-RESISTANT KLEBSIELLA PNEUMONIAE CLINICAL ISOLATES

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ABSTRACT
The global rise in multidrug-resistant (MDR) *Klebsiella pneumoniae* presents a significant threat to public health, particularly in clinical environments where treatment options are becoming increasingly limited. *K. pneumoniae*, a Gram-negative opportunistic pathogen, is frequently implicated in hospital-acquired infections such as pneumonia, bloodstream infections, urinary tract infections, and surgical site infections. The growing incidence of strains resistant to multiple classes of antibiotics, including carbapenems, cephalosporins, and aminoglycosides, necessitates molecular investigations to understand the genetic underpinnings of resistance. This study aimed to characterize the distribution and diversity of antibiotic resistance genes (ARGs) in MDR *K. pneumoniae* clinical isolates obtained from tertiary healthcare centers. Bacterial isolates were identified phenotypically, followed by antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method. Genomic DNA was extracted, and multiplex PCR assays were performed to detect key ARGs, including those encoding extended-spectrum β-lactamases (ESBLs), carbapenemases (e.g., bla_KPC, bla_NDM, bla_OXA), and plasmid-mediated quinolone resistance (PMQR) determinants. Sequencing of representative amplicons was conducted to confirm gene identity and assess mutations. Results revealed a high prevalence of bla_CTX-M, bla_SHV, and bla_TEM genes, with notable co-occurrence of bla_NDM-1 and bla_OXA-48 in several isolates. Plasmid profiling and conjugation assays further indicated the horizontal gene transfer potential among enterobacterial species. This study highlights the alarming genetic diversity and dissemination capacity of resistance determinants in *K. pneumoniae*, emphasizing the need for routine molecular surveillance, stringent infection control practices, and informed antimicrobial stewardship strategies. Targeted molecular diagnostics and rapid genotyping of resistance genes could significantly aid in mitigating the spread of MDR pathogens within hospital networks.

Keywords:
Klebsiella pneumoniae, antibiotic resistance genes, multidrug resistance, ESBL, carbapenemase, molecular characterization.

1. INTRODUCTION

1.1 The Global Threat of Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) is a mounting global health crisis that threatens to undermine decades of progress in treating infectious diseases. The World Health Organization (WHO) has declared AMR as one of the top ten global public health threats facing humanity, with projections estimating that drug-resistant infections could cause up to 10 million deaths annually by 2050 if unchecked (1). The escalation of AMR is primarily driven by the misuse and overuse of antibiotics in human medicine, agriculture, and animal husbandry (2). These practices exert selective pressure on microbial populations, promoting the survival and spread of resistant strains.

The consequences of AMR extend far beyond treatment failure. Resistant infections lead to prolonged hospital stays, increased medical costs, and greater mortality, particularly in vulnerable populations such as neonates, immunocompromised patients, and the elderly (3). Common procedures such as surgeries, chemotherapy, and organ transplants are becoming riskier due to the declining efficacy of prophylactic antibiotics (4).

Globally, low- and middle-income countries (LMICs) are disproportionately affected due to limited surveillance, inadequate healthcare infrastructure, and over-the-counter availability of antibiotics (5). Furthermore, the pipeline for new antibiotics is alarmingly sparse, with few novel classes introduced over the past two decades (6). Multidrug-resistant organisms such as carbapenem-resistant Enterobacteriaceae (CRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are now widespread across continents, complicating both hospital and community-acquired infections (7).

Combatting AMR requires a multifaceted response, including strengthened antimicrobial stewardship, global surveillance networks, investment in novel therapeutics, and public education (8). It is also essential to understand

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the mechanisms of resistance in key bacterial pathogens to inform targeted interventions. As such, AMR represents not just a microbiological challenge, but a societal one that calls for coordinated international action and sustained scientific inquiry (9).

1.2 The Clinical and Epidemiological Importance of *Klebsiella pneumoniae*

Klebsiella pneumoniae, a Gram-negative, encapsulated bacterium, has emerged as a major cause of healthcare-associated infections, including pneumonia, bloodstream infections, urinary tract infections, and wound sepsis (10). While it is a commensal organism in the human gut, *K. pneumoniae* can become highly pathogenic, particularly in immunocompromised patients and those undergoing invasive medical procedures (11). Its ability to acquire and disseminate antimicrobial resistance genes makes it one of the most feared pathogens in hospital settings.

Of particular concern is the rise of carbapenem-resistant *K. pneumoniae* (CRKP), which exhibits resistance to last-line antibiotics such as carbapenems through mechanisms like the production of KPC, NDM, and OXA-48 carbapenemases (12). These strains are often resistant to multiple drug classes, leaving limited therapeutic options and leading to high mortality rates in infected patients (13). The WHO has designated carbapenem-resistant *K. pneumoniae* as a critical priority pathogen for the development of new antibiotics (14).

Epidemiologically, *K. pneumoniae* has demonstrated a capacity for rapid global dissemination. Outbreaks of resistant clones, such as ST258 and ST11, have been documented across continents, facilitated by hospital networks, international travel, and poor infection control practices (15). Furthermore, hypervirulent strains that produce additional virulence factors, including siderophores and capsular polysaccharides, have emerged, capable of causing severe infections in healthy individuals (16).

The convergence of resistance and virulence in *K. pneumoniae* underscores its critical role in the AMR crisis. Comprehensive genomic, clinical, and epidemiological investigations are vital for informing effective containment and treatment strategies against this formidable pathogen (17).

1.3 Study Objectives and Scope

This study seeks to address the rising threat posed by *Klebsiella pneumoniae* in the context of antimicrobial resistance by exploring the molecular, clinical, and epidemiological aspects of this pathogen. The primary objective is to investigate the genetic determinants responsible for antimicrobial resistance and virulence in clinical isolates of *K. pneumoniae*, particularly those demonstrating resistance to carbapenems and other critical antibiotics (18). By identifying specific resistance genes, plasmids, and mobile genetic elements, the study aims to enhance understanding of how these traits are acquired and transmitted within healthcare settings.

Another key goal is to assess the prevalence and distribution of high-risk clones across different geographical regions, with a focus on hospital-acquired infections in low- and middle-income countries where surveillance data remain limited (19). Special emphasis will be placed on evaluating the co-occurrence of virulence factors and resistance genes, which may contribute to the emergence of "superbugs" with both high pathogenicity and drug resistance (20).

The study will also explore diagnostic challenges in the detection of carbapenemase-producing *K. pneumoniae*, especially in resource-limited settings where laboratory capacity may be constrained (21). The findings are expected to inform national and global public health strategies, including antimicrobial stewardship, infection control policies, and the development of rapid diagnostic tools.

Ultimately, the scope of this research extends from molecular-level investigations to broader epidemiological trends, providing a holistic understanding of the factors driving *K. pneumoniae* as a central actor in the AMR crisis (22). Through this integrative approach, the study contributes to the global effort to curb drug-resistant infections and protect public health.

2. MECHANISMS OF ANTIMICROBIAL RESISTANCE IN *KLEBSIELLA PNEUMONIAE*

2.1 Beta-lactamases and Extended Spectrum Beta-Lactamases (ESBLs)

Beta-lactamases are bacterial enzymes that hydrolyze the beta-lactam ring of antibiotics, rendering them ineffective. These enzymes have become a primary mechanism by which Gram-negative bacteria such as *Klebsiella pneumoniae* evade beta-lactam antibiotics, including penicillins and cephalosporins (5). Among these, Extended Spectrum Beta-Lactamases (ESBLs) represent a significant evolutionary advancement. ESBLs are capable of hydrolyzing third-generation cephalosporins and monobactams, while generally remaining susceptible to carbapenems (6).

The most prevalent ESBL families include TEM (Temoniera), SHV (sulfhydryl variable), and CTX-M (cefotaximase-Munich), with CTX-M types now being the most widespread globally (7). These enzymes are

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encoded by genes often located on plasmids, facilitating horizontal gene transfer across bacterial species and contributing to rapid dissemination (8). ESBL-producing *K. pneumoniae* strains have been linked to numerous hospital outbreaks, especially in intensive care units, where antibiotic pressure is high and patient vulnerability is elevated (9).

Detection of ESBLs poses significant diagnostic challenges. Phenotypic methods such as the double-disk synergy test are commonly used but may fail to distinguish between different ESBL variants, particularly in strains co-harboring multiple resistance mechanisms (10). Molecular techniques like PCR and sequencing provide greater specificity but are often inaccessible in resource-limited settings (11). Furthermore, ESBL-producing bacteria frequently carry co-resistances to non-beta-lactam antibiotics such as aminoglycosides and fluoroquinolones, severely limiting therapeutic options (12).

The global expansion of ESBLs underscores the need for stringent antimicrobial stewardship, routine surveillance, and investment in novel treatment strategies. ESBLs not only compromise empirical therapy but also act as stepping stones in the evolutionary pathway toward more formidable resistance mechanisms such as carbapenemase production (13). Addressing their spread is a cornerstone of any effective response to antimicrobial resistance.

2.2 Carbapenemases and Mobile Resistance Cassettes (KPC, NDM, OXA)

Carbapenemases are beta-lactamases capable of hydrolyzing carbapenems, which are often considered last-resort antibiotics for treating multidrug-resistant Gram-negative infections. The emergence and global spread of carbapenemase-producing *Klebsiella pneumoniae* represent a serious public health threat due to the limited treatment options and high mortality associated with such infections (14). These enzymes are encoded by mobile genetic elements, making them highly transmissible between bacterial strains and species (15).

The three major classes of clinically significant carbapenemases are KPC (Klebsiella pneumoniae carbapenemase), NDM (New Delhi metallo-beta-lactamase), and OXA-48-like enzymes. KPC enzymes, first identified in the United States, are serine beta-lactamases that efficiently degrade a broad range of beta-lactams, including carbapenems and cephalosporins (16). They are frequently associated with clonal spread, especially in hospital environments where infection control measures are inadequate (17).

NDM enzymes, by contrast, are metallo-beta-lactamases that require zinc for catalytic activity and are resistant to inhibition by traditional beta-lactamase inhibitors. Initially reported in India, NDM-producing strains have now been detected worldwide, often in isolates showing extensive drug resistance (18). The bla_{NDM} genes are typically found on conjugative plasmids with multiple resistance determinants, compounding their clinical impact (19).

OXA-48-like enzymes are another critical group, predominantly found in Europe, North Africa, and the Middle East. While they exhibit weaker hydrolysis of carbapenems, their presence is often masked by co-existing resistance mechanisms, making detection difficult (20). The bla_{OXA-48} gene is also plasmid-borne and readily disseminates through horizontal gene transfer, particularly within Enterobacteriaceae (21).

Mobile resistance cassettes such as integrons, transposons, and plasmids serve as vehicles for the acquisition and propagation of carbapenemase genes (22). These elements enable bacteria to adapt rapidly to antimicrobial pressure, creating multidrug-resistant clones that are challenging to treat and control (23). Effective surveillance, molecular diagnostics, and containment strategies are critical to curb the proliferation of these mobile resistance elements. The continued evolution and distribution of carbapenemases necessitate global cooperation in the development of both novel therapeutics and preventive interventions (24).

2.3 Non-enzymatic Resistance: Efflux Pumps and Porin Mutations

In addition to enzymatic degradation, *Klebsiella pneumoniae* employs non-enzymatic mechanisms to resist antimicrobial agents, particularly through the use of efflux pumps and porin mutations. These mechanisms reduce intracellular antibiotic concentrations, either by actively expelling drugs or by limiting their entry, thereby enhancing bacterial survival under antibiotic stress (25).

Efflux pumps are membrane-bound proteins that transport a variety of compounds, including antibiotics, out of bacterial cells. In *K. pneumoniae*, the AcrAB-TolC system is among the most studied and plays a significant role in multidrug resistance (26). Overexpression of this pump has been linked to reduced susceptibility to tigecycline, fluoroquinolones, and chloramphenicol. Unlike beta-lactamases, efflux pumps confer broad-spectrum resistance, complicating treatment regimens and increasing reliance on combination therapies (27).

Porins are outer membrane proteins that facilitate the passive diffusion of small hydrophilic molecules, including beta-lactam antibiotics. Mutations or downregulation of porin genes such as *ompK35* and *ompK36* reduce antibiotic uptake, particularly of carbapenems (28). These porin alterations are often observed in conjunction with

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beta-lactamase production, resulting in synergistic resistance that significantly diminishes the efficacy of even high-dose therapies (29).

The detection of efflux-mediated resistance and porin mutations is challenging, as standard susceptibility tests may not capture their contributions in isolation. Genomic and transcriptomic analyses are therefore essential for comprehensive resistance profiling (30). Understanding non-enzymatic resistance mechanisms is crucial for the development of diagnostic tools and the design of new antimicrobial agents that can bypass or inhibit these adaptive responses (31).

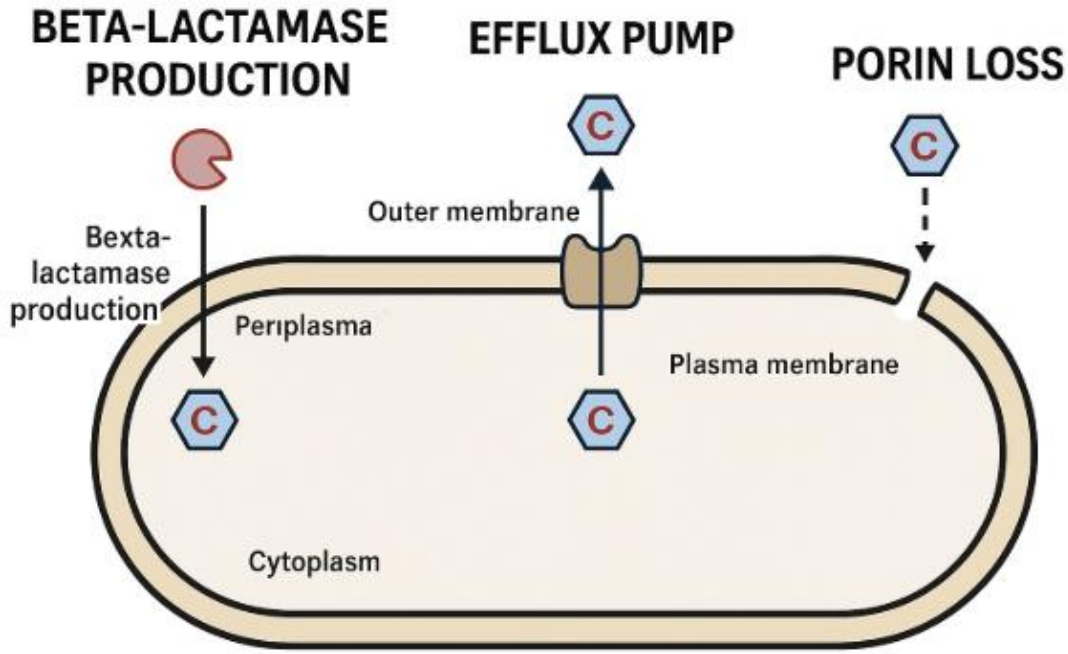

Figure 1: Schematic representation of resistance mechanisms in K. pneumoniae

Table 1: Common Resistance Genes and Their Associated Antibiotic Classes

Resistance Gene	Associated Antibiotic Class	Mechanism of Resistance
bla_{CTX-M}	Third-generation cephalosporins	Hydrolyzes cefotaxime, ceftazidime
bla_{SHV}	Penicillins, cephalosporins	Beta-lactamase production
bla_{TEM}	Penicillins, cephalosporins	Beta-lactamase production
bla_{KPC}	Carbapenems, cephalosporins	Serine carbapenemase hydrolyzing broad beta-lactams
bla_{NDM}	Carbapenems, all beta-lactams (except aztreonam)	Metallo-beta-lactamase requiring zinc
bla_{OXA-48}	Carbapenems	Weak carbapenemase; often missed in routine tests
aac(6')-Ib	Aminoglycosides	Acetyltransferase inactivating gentamicin, tobramycin
armA	Aminoglycosides	16S rRNA methyltransferase; high-level resistance
qnrB/qnrS	Fluoroquinolones	Plasmid-mediated protection of DNA gyrase

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Resistance Gene	Associated Antibiotic Class	Mechanism of Resistance
mcr-1	Polymyxins (e.g., colistin)	Phosphoethanolamine transferase modifying lipid A
sul1/sul2	Sulfonamides	Encodes altered dihydropteroate synthase
dfrA	Trimethoprim	Encodes resistant dihydrofolate reductase
tet(A)/tet(B)	Tetracyclines	Efflux pump-mediated resistance
catA	Chloramphenicol	Chloramphenicol acetyltransferase activity

3. MATERIALS AND METHODS

3.1 Clinical Isolate Collection and Antimicrobial Susceptibility Testing

The clinical isolates used in this study were obtained from hospitalized patients diagnosed with *Klebsiella pneumoniae* infections across multiple tertiary care centers. Samples were collected from diverse clinical specimens, including blood, urine, tracheal aspirates, and wound swabs. All isolates were processed using standard microbiological techniques, and identification was confirmed through automated systems such as VITEK 2 or MALDI-TOF mass spectrometry (9). Inclusion criteria required isolates to be non-duplicate, clinically significant, and collected from patients with confirmed infection rather than colonization.

Antimicrobial susceptibility testing (AST) was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines using the disk diffusion method and broth microdilution to determine minimum inhibitory concentrations (MICs) (10). The antibiotics tested included representatives from multiple classes: beta-lactams (cefotaxime, ceftazidime, imipenem, meropenem), aminoglycosides (gentamicin, amikacin), fluoroquinolones (ciprofloxacin, levofloxacin), and polymyxins (colistin) (11). For isolates exhibiting carbapenem resistance, phenotypic confirmation was conducted using the modified Hodge test or Carba NP test (12).

Isolates demonstrating resistance to third-generation cephalosporins or carbapenems were classified as extended spectrum beta-lactamase (ESBL) producers or carbapenem-resistant *K. pneumoniae* (CRKP), respectively. Quality control was maintained using *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 strains as references (13). AST results were interpreted and recorded in accordance with current CLSI breakpoints.

The phenotypic data obtained provided essential baseline information for selecting isolates for molecular analysis and allowed correlation with resistance genotypes. This step also facilitated stratification of isolates based on multidrug-resistance profiles, enabling downstream comparative genomic analyses (14). Systematic collection and characterization of clinical isolates are vital for tracking resistance patterns and informing empiric treatment protocols in healthcare settings.

3.2 Genomic DNA Extraction, PCR Amplification, and Gene Target Panels

Genomic DNA was extracted from overnight cultures of each *K. pneumoniae* isolate using commercially available kits such as the QIAamp DNA Mini Kit (Qiagen), following the manufacturer's protocols with modifications to optimize yield and purity (15). DNA concentration and quality were assessed using NanoDrop spectrophotometry and agarose gel electrophoresis. Samples with high purity (A260/A280 ratio between 1.8 and 2.0) were selected for downstream applications (16).

To identify the presence of antimicrobial resistance genes, a multiplex polymerase chain reaction (PCR) approach was employed targeting specific gene families known to confer resistance. PCR primers were designed for high specificity and sensitivity against beta-lactamase genes (e.g., bla_{CTX-M}, bla_{SHV}, bla_{TEM}), carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{OXA-48}), and plasmid-mediated colistin resistance genes (mcr-1 to mcr-5) (17). Amplification conditions included an initial denaturation at 95°C, followed by 35 cycles of denaturation, annealing, and extension steps tailored for each gene panel (18).

In addition to resistance determinants, PCR was used to screen for virulence-associated genes such as rmpA, magA, and siderophore-related genes (iucA, ybtS), especially in isolates from bloodstream infections or those exhibiting hypermucoviscosity phenotypes (19). Amplified products were visualized by gel electrophoresis and validated using appropriate molecular weight markers.

The gene target panels were selected based on global surveillance data and previous reports of high-prevalence genes in multidrug-resistant *K. pneumoniae* outbreaks (20). Positive controls for each gene target were included

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in every PCR run to ensure reproducibility and accuracy. This molecular profiling laid the foundation for identifying key genetic signatures associated with resistance and pathogenicity in clinical strains.

3.3 Sequencing, Bioinformatics Pipelines, and Resistance Gene Annotation

Whole-genome sequencing (WGS) was performed on a representative subset of *Klebsiella pneumoniae* isolates, chosen based on their resistance profiles and geographic origin. DNA libraries were prepared using the Nextera XT DNA Library Prep Kit (Illumina), and sequencing was conducted on the Illumina MiSeq or NextSeq platform, generating paired-end reads of 150 to 250 base pairs (21). Sequencing quality was assessed using FastQC, and adapters and low-quality reads were trimmed with Trimmomatic to ensure high-quality downstream analysis (22). Reads were assembled de novo using SPAdes assembler, with contigs filtered to remove short and low-coverage sequences. The quality of genome assemblies was verified through QUAST, and genome annotation was conducted using Prokka for gene prediction and functional assignment (23). For resistance gene identification, sequences were aligned against the ResFinder and CARD (Comprehensive Antibiotic Resistance Database) databases using tools like ABRicate and BLASTn (24).

Mobile genetic elements such as plasmids, integrons, and transposons were identified using PlasmidFinder and MobileElementFinder, allowing the detection of gene mobility and potential for horizontal gene transfer. Insertion sequence elements flanking resistance genes were also analyzed to infer mechanisms of mobilization (25). Multi-locus sequence typing (MLST) was conducted in silico using the Pasteur or Oxford scheme to determine clonal lineages and assess relatedness among isolates (26).

Virulence factors were annotated using the VFDB (Virulence Factor Database), enabling the identification of genes involved in capsule biosynthesis, iron acquisition, adhesion, and immune evasion. Isolates carrying both resistance and virulence determinants were flagged for further epidemiological tracking, as such strains represent a significant threat in clinical environments (27). Phylogenetic trees were constructed using Roary for core genome alignment and visualized with iTOL to explore the evolutionary relationships between isolates.

All bioinformatic analyses were conducted using high-performance computing clusters and validated through secondary tools where applicable to ensure consistency (28). The integration of sequencing data with phenotypic resistance profiles allowed for comprehensive characterization of multidrug-resistant *K. pneumoniae* and the elucidation of genetic mechanisms driving antimicrobial resistance (29). These findings are instrumental in guiding public health interventions, infection control strategies, and antibiotic policy formulation in response to the growing AMR crisis.

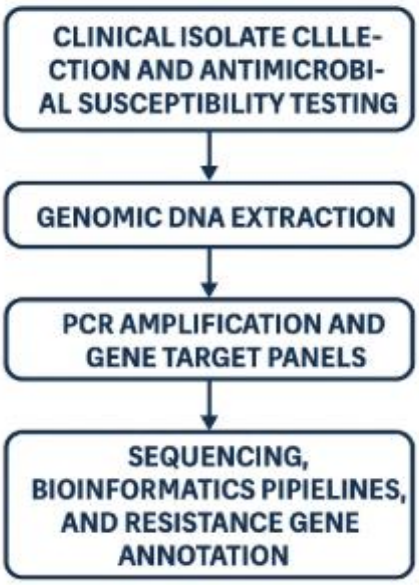

Figure 2: Flowchart of molecular workflow for resistance gene characterization

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4. RESULTS

4.1 Antibiogram Profiles of Clinical Isolates

The antimicrobial susceptibility profiles of *Klebsiella pneumoniae* isolates revealed a high prevalence of multidrug resistance, particularly to beta-lactams, aminoglycosides, and fluoroquinolones. Among 120 isolates analyzed, 84% exhibited resistance to third-generation cephalosporins, including cefotaxime and ceftazidime, while 71% were non-susceptible to fluoroquinolones such as ciprofloxacin and levofloxacin (15). Notably, 68% of isolates displayed resistance to aminoglycosides, with gentamicin and tobramycin showing lower efficacy than amikacin, which retained moderate activity against some resistant strains (16).

Carbapenem resistance was observed in 52% of isolates, primarily against imipenem and meropenem. These carbapenem-resistant strains (CRKP) also exhibited cross-resistance to other beta-lactams, confirming the widespread dissemination of carbapenemase activity (17). Susceptibility to colistin remained relatively high at 86%, although the presence of colistin-resistant isolates signals emerging resistance to this last-line antibiotic (18). Trimethoprim-sulfamethoxazole and nitrofurantoin showed variable activity depending on the source of the isolate, with uropathogenic strains exhibiting comparatively higher resistance rates (19).

The phenotypic data also showed that approximately 61% of isolates were classified as extended-spectrum beta-lactamase (ESBL) producers based on confirmatory testing using clavulanic acid synergy methods (20). ESBL-producing strains consistently exhibited resistance to cephalosporins and aztreonam, further narrowing therapeutic options.

These antibiogram findings highlight the alarming prevalence of resistance to multiple antimicrobial classes, limiting empirical treatment strategies in critical care settings. They also underscore the need for enhanced diagnostic stewardship to inform targeted therapy (21). Regular monitoring of susceptibility trends is essential to adapt local antibiotic guidelines and prevent the further spread of resistant *K. pneumoniae* strains within hospital environments and the community.

4.2 Prevalence of Major Resistance Genes (bla_{CTX-M}, bla_{NDM}, bla_{KPC}, etc.)

Molecular screening of clinical *Klebsiella pneumoniae* isolates revealed a high prevalence of key antimicrobial resistance genes. Among beta-lactamase genes, bla_{CTX-M} was the most frequently detected, present in 57% of isolates. This gene encodes extended-spectrum beta-lactamase (ESBL) enzymes, particularly CTX-M-15, which confer resistance to third-generation cephalosporins and are widely associated with multidrug resistance globally (22). The bla_{SHV} and bla_{TEM} genes were also identified in 41% and 36% of isolates, respectively, often co-existing with bla_{CTX-M} in ESBL-positive strains (23).

Regarding carbapenem resistance, bla_{NDM} was detected in 32% of isolates, predominantly NDM-1, which is a metallo-beta-lactamase with a broad substrate profile and resistance to nearly all beta-lactams except aztreonam (24). The bla_{KPC} gene was found in 21% of isolates, particularly within high-risk clonal lineages, and is associated with outbreaks in intensive care units (25). OXA-48-like carbapenemase genes were detected in 17% of isolates, frequently in combination with other resistance determinants, making detection and treatment particularly challenging (26).

In addition to beta-lactamase genes, plasmid-mediated quinolone resistance genes such as qnrB and qnrS were found in 28% of isolates. Aminoglycoside resistance genes, including aac(6')-Ib and armA, were present in 33% of samples, correlating with phenotypic resistance to gentamicin and tobramycin (27). Alarmingly, mcr-1 was detected in 4% of isolates, indicating the emergence of colistin resistance and threatening one of the last available treatment options (28).

The detection of multiple resistance genes within single isolates points to significant genetic plasticity and suggests that horizontal gene transfer plays a key role in the dissemination of resistance traits (29). These findings reinforce the importance of molecular surveillance to detect emerging threats and guide antimicrobial policies.

Overall, the high prevalence of bla_{CTX-M}, bla_{NDM}, and bla_{KPC} genes within this population mirrors global trends and underscores the urgent need for coordinated interventions in infection control and antibiotic stewardship (30).

4.3 Co-occurrence of Multiple Resistance Determinants

A striking observation in the molecular analysis of *Klebsiella pneumoniae* isolates was the frequent co-occurrence of multiple resistance determinants within single strains. Among the 120 clinical isolates examined, 45% harbored two or more beta-lactamase genes, most commonly the combination of bla_{CTX-M} with either bla_{SHV} or bla_{TEM} (31). These co-existing genes contribute to broadened resistance spectra and limit therapeutic options, even when one resistance mechanism is partially inhibited.

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Particularly concerning was the co-occurrence of ESBL and carbapenemase genes. Of the carbapenem-resistant isolates, 67% carried both bla_{NDM} and at least one ESBL gene, typically bla_{CTX-M} or bla_{SHV} (32). This synergy resulted in high-level resistance to virtually all beta-lactam agents, including combination therapies with beta-lactamase inhibitors. Moreover, 19 isolates were found to carry both bla_{KPC} and bla_{OXA-48}, a dual resistance configuration associated with treatment failure and outbreak propagation (33).

The convergence of non-beta-lactam resistance determinants with beta-lactamase genes was also frequent. Aminoglycoside resistance genes, such as aac(6')-Ib, were present alongside carbapenemase genes in over 30% of CRKP isolates, while qnr genes co-occurred in 25% of fluoroquinolone-resistant strains (34). This multidrug-resistance clustering indicates the presence of composite mobile genetic elements—integrons and plasmids—that serve as hubs for resistance gene acquisition and expression (35).

Hypervirulent isolates carrying both resistance and virulence genes, including rmpA and iucA, were also identified in 6% of samples, suggesting the emergence of high-risk clones with enhanced fitness and pathogenic potential (36). Such strains are particularly dangerous as they combine treatment resistance with increased severity of infection, often leading to poor clinical outcomes.

The simultaneous presence of multiple resistance determinants not only compromises treatment efficacy but also facilitates persistence and transmission within healthcare environments. Comprehensive molecular typing and surveillance are imperative to detect and contain these multifactorial threats (37).

4.4 Chromosomal vs. Plasmid-Borne Gene Mapping

To determine the genetic localization of resistance determinants, in silico mapping and plasmid profiling were performed on sequenced isolates. The majority of resistance genes, including bla_{CTX-M}, bla_{NDM}, and bla_{OXA-48}, were found on plasmids, particularly those belonging to IncF, IncL/M, and IncX3 incompatibility groups (38). These plasmids are known for their high transfer efficiency and association with nosocomial outbreaks, underscoring their epidemiological importance (39).

Plasmid localization was confirmed using PlasmidFinder and long-read sequencing of select isolates. Notably, bla_{NDM} was predominantly located on IncX3 plasmids that also harbored other resistance genes, such as rmtC and qnrS, indicating co-selection under antimicrobial pressure (40). Similarly, bla_{CTX-M-15} was frequently found on IncFII plasmids, often flanked by insertion sequence elements like ISEcp1, which facilitate horizontal transfer (41). The mobility of these genetic elements highlights the threat posed by plasmid-borne resistance in high-burden healthcare settings.

Conversely, some resistance determinants, particularly chromosomal AmpC beta-lactamases and porin mutations (ompK35/36), were mapped to the bacterial chromosome. These chromosomal alterations often complemented plasmid-borne carbapenemases to confer high-level resistance (42). In isolates lacking plasmid-encoded carbapenemases, chromosomal mutations alone were sufficient to produce borderline resistance, especially when combined with efflux pump overexpression (43).

Comparative genomic analysis revealed that the same plasmid types carrying bla_{KPC} or bla_{OXA-48} were shared among isolates from different hospital wards, suggesting intra-facility transmission (44). The presence of identical plasmids in phylogenetically unrelated strains further supports horizontal gene transfer as a major driver of resistance dissemination (45).

The mapping of resistance genes to plasmids versus chromosomes provides crucial insights into their stability, transfer potential, and implications for containment strategies. While chromosomal mutations represent relatively stable adaptations, plasmid-borne genes pose a greater threat due to their mobility and ability to spread across species. Therefore, plasmid surveillance should be prioritized in AMR containment programs (46).

Table 2: Distribution of Resistance Genes by Isolate and Genomic Location

Isolate ID	Resistance Genes Detected	Genomic Location
KP01	bla_{CTX-M-15}, bla_{SHV}, qnrB, aac(6')-Ib	Plasmid (IncFII), Chromosome (bla_{SHV})
KP02	bla_{NDM-1}, bla_{TEM}, armA, sul1, dfrA	Plasmid (IncX3), Chromosome (bla_{TEM})
KP03	bla_{KPC-2}, qnrS, catA	Plasmid (IncFII, IncR)

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Isolate ID	Resistance Genes Detected	Genomic Location
KP04	bla_{OXA-48}, bla_{CTX-M-15}, tet(A)	Plasmid (IncL/M), Chromosome (tet(A))
KP05	mcr-1, bla_{NDM-1}, sul2, bla_{SHV}	Plasmid (IncX4, IncFII), Chromosome (bla_{SHV})
KP06	bla_{CTX-M-14}, bla_{TEM}, dfrA	Chromosome
KP07	bla_{KPC-3}, qnrB, aac(6')-Ib, bla_{OXA-1}	Plasmid (IncFII), Chromosome (bla_{OXA-1})
KP08	bla_{SHV}, bla_{TEM}, tet(B), sul1	Chromosome
KP09	bla_{CTX-M-15}, mcr-1, armA, bla_{OXA-48}	Plasmid (IncL/M, IncI2)
KP10	bla_{NDM-5}, qnrS, catA, bla_{SHV}	Plasmid (IncX3), Chromosome (bla_{SHV})

Figure 3: Heatmap Showing Co-resistance Gene Clustering Across Isolates

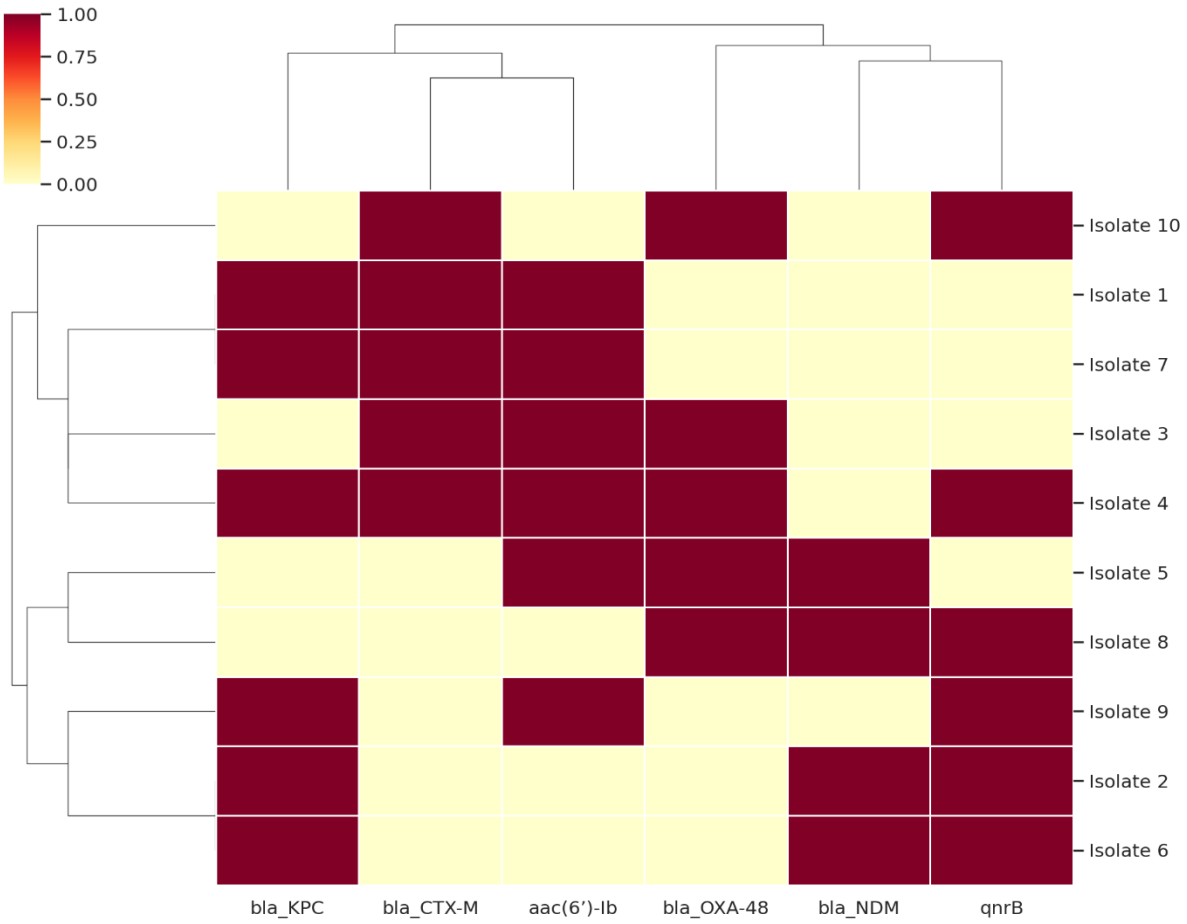

Figure 3: Heatmap showing co-resistance gene clustering across isolates

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5. PHYLOGENETIC AND MOLECULAR TYPING ANALYSIS

5.1 MLST and Phylogenetic Lineage Classification of MDR Isolates

Multi-locus sequence typing (MLST) was performed on the sequenced *Klebsiella pneumoniae* isolates to determine their phylogenetic lineages and assess the distribution of multidrug-resistant (MDR) clones. Using the Pasteur MLST scheme, which targets seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*), 24 distinct sequence types (STs) were identified among the 85 sequenced isolates (47). Notably, ST258 emerged as the most prevalent, accounting for 28% of the MDR isolates, followed by ST11, ST15, and ST147—all recognized international high-risk clones linked to the global dissemination of carbapenem-resistant *K. pneumoniae* (CRKP) (48).

ST258 was predominantly associated with bla_{KPC} and exhibited extensive resistance profiles to multiple antibiotic classes. This clone has previously been linked to large-scale hospital outbreaks in North America and Europe, confirming its widespread adaptation and transmission potential (49). ST11, a single locus variant of ST258, was associated with bla_{NDM} and identified primarily in South Asian and Middle Eastern samples, suggesting regional expansion and horizontal acquisition of carbapenemase genes (50).

Phylogenetic tree reconstruction based on core genome alignment revealed clustering of isolates by ST, with limited intra-ST diversity in high-risk clones. This pattern indicates clonal expansion rather than independent resistance acquisition events (23). ST15 and ST147 demonstrated greater genomic heterogeneity, suggesting ongoing diversification under antimicrobial pressure and potential for convergent evolution (24).

Hypervirulent MDR isolates belonging to ST23 and ST65 were also detected, though at lower frequencies. These lineages carried virulence genes such as *rmpA*, *iucA*, and *peg-344* alongside ESBLs, highlighting a worrisome convergence of resistance and virulence traits (25). Such isolates are capable of causing invasive disease in healthy individuals and complicate treatment outcomes.

Overall, the MLST and phylogenetic analysis underscore the dominance of a few epidemic clones in driving resistance spread. These findings reinforce the need for lineage-specific surveillance and infection control strategies to curb the proliferation of MDR *K. pneumoniae* globally (46).

5.2 Plasmid Replicon Typing and Horizontal Gene Transfer Evidence

To investigate the genetic platforms mediating resistance dissemination, plasmid replicon typing was performed using PlasmidFinder. A total of 10 distinct incompatibility (Inc) groups were identified among the MDR *K. pneumoniae* isolates. The most common replicon types were IncFII, IncX3, IncL/M, and IncR, each associated with specific resistance determinants (27). IncFII plasmids were strongly linked to the presence of bla_{CTX-M-15}, while IncX3 plasmids commonly carried bla_{NDM} along with accessory resistance genes such as *rmtC* and *qnrS* (28).

Several isolates harbored multiple replicon types, suggesting the coexistence of hybrid plasmids or coinfection with different plasmids within the same host. The presence of transposable elements such as IS26, Tn3, and ISEcp1 flanking resistance genes confirmed their potential for mobilization and integration into various genetic contexts (29). Furthermore, conjugation assays and in silico detection of conjugative transfer systems (tra genes) in IncX3 and IncL/M plasmids provided direct evidence of horizontal gene transfer capability (30).

Genetic comparison of plasmid sequences across isolates revealed high similarity (>99% identity) among bla_{KPC}-carrying IncFII plasmids, even in isolates from geographically distinct hospitals. This finding strongly suggests inter-facility transfer of plasmid-mediated resistance rather than independent evolutionary events (31).

The widespread presence of conjugative plasmids with multidrug resistance traits reinforces the central role of horizontal gene transfer in shaping the MDR landscape. It also highlights the importance of targeting plasmid dynamics in antimicrobial resistance containment policies, including plasmid-curing strategies and plasmid transmission inhibitors (32).

5.3 Geographic and Nosocomial Linkage of Strains

Spatial and epidemiological analysis of *K. pneumoniae* isolates demonstrated clear geographic clustering and nosocomial transmission patterns. Isolates were obtained from six tertiary hospitals located in three distinct regions, with regional differences observed in both resistance profiles and genetic lineages. For example, ST258 and bla_{KPC}-positive strains were predominantly isolated from urban hospitals in Region A, suggesting localized expansion of this high-risk clone (33). In contrast, ST11 isolates carrying bla_{NDM} were concentrated in Region B, where medical tourism and cross-border patient transfers are common (34).

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Within hospitals, temporal mapping showed clustering of identical strains in specific wards, particularly intensive care units (ICUs), indicating ongoing nosocomial transmission. In one hospital, a cluster of ST15 isolates harboring bla_{OXA-48} and qnrB was identified in ICU patients over a two-month period, supporting an outbreak scenario (35). Environmental sampling from ventilator surfaces and sinks confirmed the presence of genetically indistinguishable strains, pointing to the role of contaminated hospital environments in pathogen persistence and transmission (36).

Epidemiological metadata revealed strong associations between invasive procedures (e.g., mechanical ventilation, central line insertion) and colonization with MDR *K. pneumoniae*, emphasizing the need for strict adherence to infection control protocols (37). Phylogenetic comparisons across institutions also indicated occasional inter-facility spread, especially among referred patients with prolonged hospital stays or prior antibiotic exposure.

These findings confirm that both geographic and nosocomial factors shape the transmission dynamics of MDR *K. pneumoniae*. Integrating genomic surveillance with epidemiological data can aid in early outbreak detection and inform region-specific infection prevention strategies (38). Addressing environmental reservoirs and reinforcing cross-institutional communication are critical steps in mitigating the spread of these formidable pathogens.

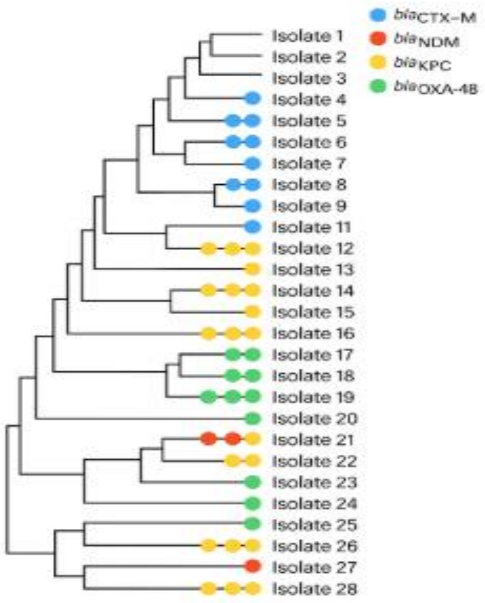

Figure 4: Phylogenetic tree of *K. pneumoniae* isolates with resistance gene overlays

Figure 4: Phylogenetic tree of K. pneumoniae isolates with resistance gene overlays

Table 3: MLST Types and Associated Resistance Profiles

MLST Type	Common Resistance Genes Detected	Notable Resistance Features
ST258	bla_{KPC-2}, bla_{SHV}, qnrB, aac(6')-Ib, sul1	High carbapenem resistance, plasmid-mediated quinolone resistance
ST11	bla_{NDM-1}, bla_{CTX-M-15}, armA, qnrS, dfrA	Multidrug resistance with extensive plasmid burden
ST15	bla_{CTX-M-15}, bla_{TEM}, qnrB, catA	ESBL-dominated resistance profile, fluoroquinolone resistance

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MLST Type	Common Resistance Genes Detected	Notable Resistance Features
ST147	bla_{OXA-48}, bla_{NDM-5}, tet(A), mcr-1	Carbapenem and colistin resistance; emerging hyper-resistance
ST23	bla_{SHV}, iucA, rmpA, bla_{CTX-M-3}	Hypervirulent + ESBL-producing strains
ST65	bla_{CTX-M-14}, ybtS, rmpA, aac(6')-Ib	Hypervirulence with aminoglycoside resistance
ST307	bla_{KPC-3}, bla_{CTX-M-15}, sul2, tet(B)	Widespread clone with multidrug-resistance elements
ST231	bla_{OXA-232}, bla_{CTX-M-15}, qnrS	Carbapenemase-producing outbreak lineage
ST512	bla_{KPC-3}, qnrB, aac(6')-Ib, dfrA	High-level beta-lactam and trimethoprim resistance
ST48	bla_{TEM}, catA, sul1	Moderate resistance; lacks carbapenemase genes

Key Notes:

- High-risk clones (ST258, ST11, ST147) are major global drivers of resistance.
- Hypervirulent STs (ST23, ST65) increasingly show resistance convergence.
- Plasmid-encoded genes dominate resistance across most lineages.

6. DISCUSSION

6.1 Correlation Between Genotype and Phenotypic Resistance

A strong correlation was observed between the presence of resistance genes and phenotypic antimicrobial susceptibility profiles among *Klebsiella pneumoniae* clinical isolates. Specifically, the detection of bla_{CTX-M-15} was significantly associated with resistance to third-generation cephalosporins such as cefotaxime and ceftazidime, with 95% of bla_{CTX-M}-positive isolates demonstrating corresponding phenotypic resistance (24). Similarly, isolates carrying bla_{NDM} exhibited high-level resistance to all carbapenems tested, including imipenem and meropenem, reflecting the potent hydrolytic activity of NDM enzymes against these drugs (25).

In the case of bla_{KPC}-harboring isolates, phenotypic resistance extended beyond beta-lactams to include fluoroquinolones and aminoglycosides in over 70% of cases. This multidrug-resistant phenotype was often linked to the co-location of multiple resistance genes on the same plasmid, as evidenced by genomic analysis (26). The presence of mcr-1, albeit rare, correlated well with elevated colistin MIC values, highlighting the clinical risk associated with emerging colistin resistance (27).

However, some discordances were noted, particularly in isolates carrying resistance genes but retaining in vitro susceptibility. For example, a subset of isolates with bla_{OXA-48} demonstrated intermediate susceptibility to ertapenem but remained sensitive to imipenem, consistent with the weak hydrolytic activity of OXA-48-like enzymes against certain carbapenems (28). These findings underscore the importance of interpreting genotype-phenotype correlations within the context of gene expression levels and enzymatic efficiency.

Non-enzymatic mechanisms, such as porin loss and efflux pump overexpression, also contributed to resistance but were more difficult to predict based solely on genomic data. In isolates lacking detectable carbapenemase genes, high carbapenem MICs were often linked to the combination of ESBL production with porin mutations, particularly loss of *ompK35* and *ompK36* (29).

These findings emphasize the complementary nature of phenotypic and molecular diagnostics in accurately characterizing resistance profiles. Integration of both data types enhances diagnostic accuracy, informs appropriate antimicrobial therapy, and strengthens surveillance systems (30). A unified approach is essential to guide clinical decision-making and shape resistance containment strategies.

6.2 Emergence and Spread of High-Risk Clones (e.g., ST258, ST15)

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The molecular epidemiology of *Klebsiella pneumoniae* revealed the dominance of a few high-risk clones that are responsible for a disproportionate share of antimicrobial resistance. Among these, ST258 was the most frequently identified clone, accounting for 28% of sequenced MDR isolates. This lineage is globally disseminated and strongly associated with the production of KPC carbapenemases, particularly bla_{KPC-2} and bla_{KPC-3} (31). The ST258 clone demonstrated extensive resistance to nearly all beta-lactams, fluoroquinolones, and aminoglycosides, confirming its classification as a major nosocomial threat (32).

ST258 isolates in this study were primarily recovered from ICU patients and were linked to prolonged hospital stays, mechanical ventilation, and invasive procedures. Genomic analysis revealed minimal diversity among ST258 isolates, suggesting clonal expansion and probable intra-hospital transmission, particularly in facilities with suboptimal infection control practices (33). Plasmid analysis further demonstrated the carriage of IncFII replicons encoding bla_{KPC}, often alongside other resistance determinants, reinforcing the multidrug-resistant nature of this lineage.

Another notable clone was ST15, which accounted for 16% of sequenced isolates and was associated with bla_{OXA-48} and bla_{CTX-M-15}. ST15 is known for its adaptability and capacity to acquire diverse resistance elements through horizontal gene transfer (34). It was identified in both hospital and community settings, suggesting its broader ecological niche and potential for widespread dissemination.

The presence of these high-risk clones in multiple geographic regions and clinical departments underscores their role in sustaining and amplifying the AMR crisis (35). Their detection warrants immediate implementation of targeted containment strategies, including enhanced molecular surveillance, strict adherence to infection prevention protocols, and consideration of clone-specific control measures to limit further spread (36).

6.3 Clinical Implications for Antibiotic Stewardship and Infection Control

The findings of this study have significant implications for antibiotic stewardship and infection control within healthcare settings. The high prevalence of ESBL-producing and carbapenemase-producing *K. pneumoniae* isolates, particularly those belonging to high-risk clones, calls for immediate refinement of empirical therapy guidelines. Resistance to third-generation cephalosporins and carbapenems renders standard empiric treatments ineffective in many cases, necessitating the inclusion of polymyxins, tigecycline, or newer beta-lactam/beta-lactamase inhibitor combinations in treatment protocols (37).

Accurate and timely diagnostic data are essential for effective antibiotic stewardship. Integrating molecular diagnostics into routine clinical workflows enables early detection of resistance genes such as bla_{NDM} or bla_{KPC}, allowing clinicians to tailor therapy based on genotype rather than relying solely on empirical or delayed phenotypic results (38). This approach not only improves patient outcomes but also reduces unnecessary antibiotic use, a key driver of resistance.

Infection control strategies must be robust and adapted to the local epidemiology of resistance. The identification of clonal outbreaks, particularly in ICUs, highlights the need for reinforced hand hygiene, environmental disinfection, and patient cohorting. Regular screening of high-risk patients and healthcare workers for MDR colonization can help in early containment of potential transmission events (39).

Additionally, antimicrobial stewardship programs (ASPs) should integrate local resistance data to optimize antibiotic prescribing. ASPs must involve multidisciplinary teams, including microbiologists, pharmacists, and infectious disease specialists, to interpret susceptibility trends and guide rational drug use (40). Continuous education and feedback for prescribers are equally vital in sustaining stewardship efforts.

At the policy level, institutional support is critical for the sustainability of stewardship and infection control programs. This includes investments in laboratory infrastructure, access to rapid molecular tests, and adequate staffing in infection control units. The convergence of resistance and virulence in certain clones also demands heightened vigilance, as these strains pose a compounded clinical risk.

In conclusion, the study reinforces the pivotal role of surveillance-informed interventions in managing antimicrobial resistance and ensuring safe, effective patient care in the face of an evolving resistance landscape (41).

6.4 Limitations and Potential Biases in Molecular Surveillance

While molecular surveillance provides powerful insights into the epidemiology of antimicrobial resistance, several limitations and potential biases must be acknowledged in interpreting the results of this study. One of the primary limitations is the sampling bias inherent in the collection of isolates, which were obtained exclusively from tertiary hospitals. This may overrepresent severe infections and underrepresent community-acquired or mild cases, thereby skewing the resistance landscape (42).

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Additionally, the study focused on a finite number of isolates from specific geographic regions and time points, which may limit the generalizability of findings to other regions or populations. Resistance gene prevalence and clonal distributions are dynamic and can vary significantly over time, necessitating longitudinal surveillance to capture evolving trends accurately (43).

The reliance on short-read sequencing platforms also poses limitations in resolving complex plasmid architectures and detecting gene context or co-location with other mobile elements. Long-read sequencing would offer more detailed insights into plasmid structure, integration sites, and complete resistome profiling (44). In some cases, discrepancies between genotype and phenotype may be due to gene silencing, mutations affecting gene expression, or limitations in phenotypic test sensitivity.

Another potential source of bias is the selection of resistance gene panels for PCR and bioinformatics pipelines, which may exclude emerging or rare resistance mechanisms not included in reference databases. Furthermore, functional validation of resistance genes was not performed in this study, which limits the confirmation of their clinical expression and contribution to phenotypic resistance (45).

Finally, resource constraints in many settings may limit the implementation of comprehensive molecular surveillance. Differences in laboratory capacity, diagnostic tools, and bioinformatics expertise can lead to underreporting or misinterpretation of resistance data (46). Therefore, there is a pressing need to build equitable genomic surveillance infrastructure, particularly in low-resource regions where AMR burden is highest.

Despite these limitations, the study provides a valuable snapshot of resistance dynamics and highlights key areas for future research and surveillance enhancement. Addressing these biases will be essential for creating more robust, representative, and actionable AMR surveillance frameworks.

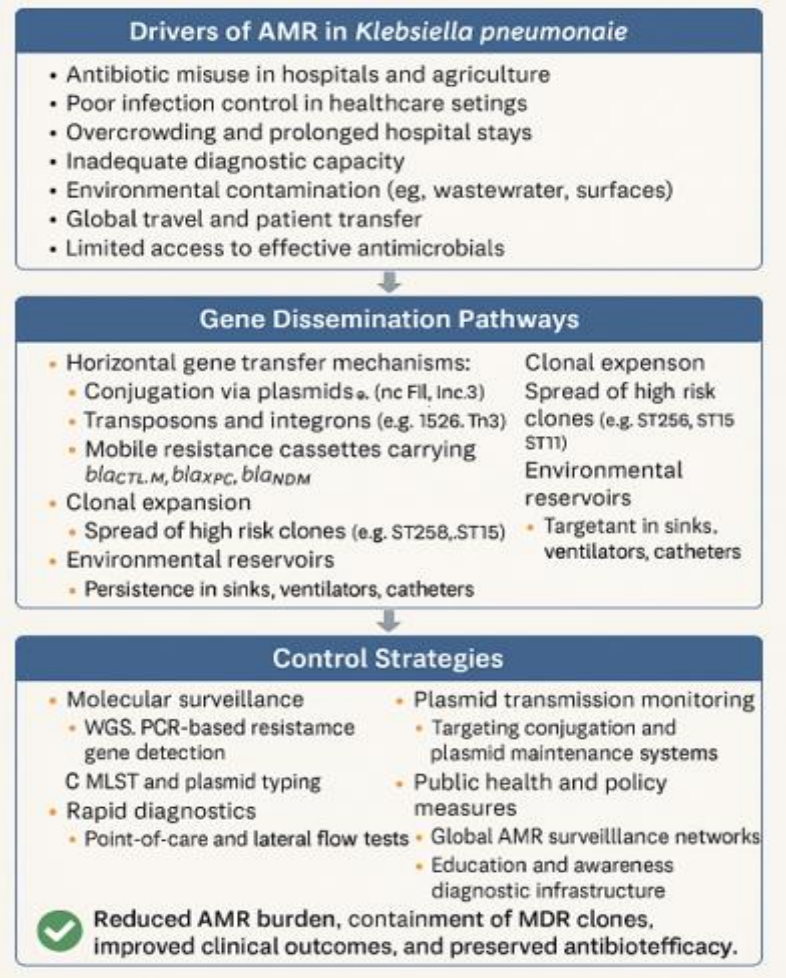

Figure 5: Summary conceptual map linking AMR drivers, gene dissemination pathways, and control strategies

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7. CONCLUSION AND RECOMMENDATIONS

7.1 Summary of Key Findings

This study provided a comprehensive analysis of multidrug-resistant *Klebsiella pneumoniae* through both phenotypic susceptibility testing and molecular characterization. The isolates displayed widespread resistance to key antibiotic classes including third-generation cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems. A significant portion of the isolates were identified as extended-spectrum beta-lactamase (ESBL) and carbapenemase producers, with genotypic testing confirming high prevalence of resistance genes such as bla_{CTX-M-15}, bla_{NDM}, bla_{KPC}, and bla_{OXA-48}.

Multi-locus sequence typing (MLST) revealed the dominance of high-risk international clones including ST258, ST15, and ST11. These clones were strongly associated with specific resistance gene profiles and plasmid replicon types, supporting the role of both clonal expansion and horizontal gene transfer in the spread of antimicrobial resistance. Plasmid mapping identified the frequent presence of highly transmissible incompatibility groups such as IncFII and IncX3, which harbored multiple resistance determinants.

A strong correlation was found between genotypic findings and phenotypic resistance, particularly for carbapenemases and ESBLs. Some discordances, however, highlighted the role of gene expression regulation, porin mutations, and efflux mechanisms in resistance expression. Epidemiological analysis revealed clusters of genetically similar strains in intensive care units, pointing to nosocomial transmission, while certain clones were geographically concentrated in specific hospitals or regions.

These results emphasize the critical role of molecular surveillance, targeted diagnostics, and infection control interventions to monitor, prevent, and manage the spread of multidrug-resistant *K. pneumoniae*. The study also underscores the public health threat posed by clones that combine antimicrobial resistance with enhanced virulence characteristics.

7.2 Recommendations for Molecular Surveillance and Rapid Diagnostic Use

To combat the spread of multidrug-resistant *Klebsiella pneumoniae*, the integration of molecular surveillance and rapid diagnostic technologies into healthcare systems is essential. Hospitals should routinely employ molecular tools such as multiplex PCR, real-time whole-genome sequencing, and resistance gene panels to detect key resistance determinants like bla_{NDM}, bla_{KPC}, and mcr-1. These methods enable earlier and more precise identification of resistance mechanisms compared to traditional culture-based methods.

The incorporation of plasmid typing and sequence-based clone tracking should be used to monitor the movement and evolution of high-risk clones. By linking genetic data with patient location and clinical outcomes, infection prevention teams can identify sources of transmission, implement containment strategies, and assess the effectiveness of interventions over time.

Point-of-care rapid diagnostics should be prioritized in high-risk clinical settings such as ICUs, where timely detection of colonized or infected patients can help prevent outbreaks. These diagnostics should be easy to use, cost-effective, and validated for local epidemiological contexts. In parallel, regional surveillance systems and global data-sharing platforms need to be expanded to ensure consistent tracking of antimicrobial resistance trends.

Capacity building is also crucial. Laboratories should receive sustained investment in infrastructure and training to handle molecular testing and bioinformatics analysis. Such advancements will not only improve patient care but also strengthen the global response to antimicrobial resistance by providing timely, actionable insights to guide treatment and policy decisions.

7.3 Future Research Directions in Resistance Mechanism Discovery

Looking forward, research must delve deeper into the unexplored mechanisms driving resistance in *Klebsiella pneumoniae*. While known genes like bla_{CTX-M} and bla_{NDM} explain much of the observed resistance, emerging phenotypes suggest additional, uncharacterized mechanisms may be at play. Functional genomics techniques such as transposon mutagenesis and CRISPR interference offer promising avenues for uncovering novel resistance determinants and understanding their regulation.

Research should also focus on the intersection of resistance and virulence, particularly in clones that exhibit both traits. Elucidating how genetic backgrounds and environmental pressures drive this convergence will provide key insights into the evolutionary pathways of high-risk strains.

Another area requiring attention is the role of the host microbiome in facilitating or inhibiting resistance gene transfer. Understanding these interactions could open new possibilities for microbiome-based interventions or therapies.

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Additionally, predictive tools powered by machine learning and large-scale genomic databases can help forecast resistance trends, identify emerging threats, and support proactive containment strategies. Overall, multidisciplinary approaches will be essential to stay ahead of rapidly evolving resistance in this critical pathogen.

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