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Drivers of Antimicrobial Resistance in *Klebsiella pneumoniae*

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"Looking backwards might be the only way to move forward." *— The Manuscript*

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LIST OF ACRONYMS

AMR	Antimicrobial Resistance
GDP	Global Domestic Product
ESBL	Extended-Spectrum Beta-Lactamase
PBP	Penicillin-Binding Proteins
MDR	Multidrug-Resistant
WHO	World Health Organization
BPPL	Bacterial Priority Pathogen List
CRE	Carbapenem-Resistant Enterobacteriaceae
HGT	Horizontal Gene Transfer
pCONJ	Conjugative Plasmids
pdCONJ	Decayed Conjugative Plasmids
pMOB	Mobilizable Plasmids
pNT	Non-Transmissible Plasmids
T4SS	Type IV Secretion System
oriT	Origin of Transfer
oriV	Origin of Replication
ARG	Antibiotic Resistance Genes
Inc	Incompatibility
PTU	Plasmid Taxonomic Units
NCBI	National Center for Biotechnology Information
RefSeq	Reference Sequence
PanACoTA	PANgenome with Annotations, COre identification, Tree and corresponding Alignments
KpSC	Klebsiella pneumoniae Species Complex
MLST	Multilocus Sequence Typing
ST	Sequence Type
AIC	Akaike Information Criterion
ICC	Intraclass Correlation Coefficient

ABSTRACT

Antimicrobial resistance (AMR) is a growing public health threat with wide-ranging implications. A major concern is the increasing prevalence of beta-lactamase enzymes. Beta-lactamases particularly extended-spectrum beta-lactamases (ESBLs) and carbapenemases, have become a central focus in the fight against AMR due to their rapid emergence and global spread. *Klebsiella pneumoniae* is a major driver of this resistance because of its ability to acquire and disseminate beta-lactamase genes through plasmids.

This study investigated the global distribution of beta-lactamase-carrying plasmids in *K. pneumoniae*, using 2,074 complete genomes from NCBI RefSeq and their associated metadata from Genbank. Resistance genes were detected using AMRFinderPlus, while plasmids types and features were identified using a new in-house typing framework.

The majority of antibiotic resistance genes (68.7%) were plasmid-borne, with beta-lactamases showing the highest gene diversity and frequency. Among 7,147 plasmids identified, 2,827 (39.6%) carried at least one beta-lactamase genes and most of which were conjugative. A total of 224 plasmid types were identified and 95 were linked to beta-lactamase carriage. Few plasmid types exclusively carried either ESBL or cabapenemase genes, while most were shared across resistance subtypes.

Statistical analyses revealed strong associations between plasmid types and resistance subgroups, with significant geographic structuring. For instance, plasmid type 333 was exclusively linked to carbapenemase carriage in Asia, while 276 was more predominant in Europe. Logistic regression models with interaction terms confirmed that plasmid–resistance associations varied significantly by continent. Sequence alignment of the identified carbapenemase-carrying plasmids in Asia (333) and Europe (276) showed distinct genetic backbones, supporting the idea that different plasmid lineages are driving resistance in different regions.

These findings highlight the role of plasmids in the global dissemination of beta-lactamase genes. Understanding which plasmid types are common in each region could help guide more targeted interventions in the ongoing fight against AMR.

KEY WORDS: Antimicrobial Resistance, Klebsiella pneumoniae, Beta-Lactamase, Plasmids

RÉSUMÉ

La résistance aux antimicrobiens (RAM) est un enjeu majeur de santé publique, avec des conséquences majeures à l'échelle mondiale. Notamment, l'émergence et la propagation d'antibiotiques particuliers tels que les bêta-lactamases à large spectre (ESBL) et les carbapénémases, sont au cœur de la lutte contre la RAM. *Klebsiella pneumoniae*, de par sa capacité à acquérir et disséminer des plasmides porteurs de gènes de bêta-lactamase, joue un rôle clé dans cette désamination.

Dans cette étude, nous avons étudié la distribution mondiale des plasmides porteurs de gènes de bêta-lactamase chez *K. pneumoniae*. Pour cela, nous avons analysé 2 074 génomes complets issus de NCBI RefSeq ainsi que les métadonnées associées. Les gènes de résistance ont été détectés avec AMRFinderPlus, et les plasmides portés par ces bactéries ont été classés en famille.

Parmi les gènes de résistance détectés dans les génomes, la majorité (68,7 %) étaient portés par des plasmides, et les gènes codant des bêta-lactamases étaient de loin les plus divers et fréquents. Sur les 7 147 plasmides de notre jeu de données, 2 827 (39,6 %) portaient au moins un gène de bêta-lactamase, et la plupart de ces plasmides étaient conjugatifs. Sur 224 familles de plasmides identifiés, 95 étaient liées à la présence de bêta-lactamases. Tandis que la plupart de ces familles étaient associées à la fois aux ESBL et aux carbapénémases, certaines familles étaient exclusivement associées exclusivement à l'une de ces beta-lactamases.

Les analyses statistiques ont révélé des associations fortes entre familles de plasmides et sous-groupes de résistance, avec une structuration géographique nette. Par exemple, la famille 333 était exclusive à l'Asie, tandis que le 276 dominait en Europe, bien que toutes deux portent le même gène de résistance aux carbapénèmases. Ces différences ont été confirmées par des modèles de régression logistique et des alignements de séquences.

Ces résultats soulignent le rôle central des plasmides dans la diffusion mondiale des gènes de bêta-lactamase et l'importance d'approches de surveillance adaptées et spécialisées au contexte régional.

MOTS-CLÉS: Résistance aux antimicrobiens, *Klebsiella pneumoniae*, Bêta-lactamase, Plasmides

INTRODUCTION

Global Burden of Antimicrobial Resistance

Antimicrobials are substances used to treat infections caused by disease-causing microbes such as bacteria, viruses, fungi, and parasites. Among them, antibiotics (also known as antibacterials), specifically used to treat bacterial infections, are the most familiar and widely used. Their discovery is noted to have been one of the most important discoveries of modern medicine, revolutionizing clinical practice, by saving countless lives and making high-risk procedures such as surgeries, chemotherapy and organ transplants much safer to perform (1).

However, this success is now under threat due to the growing problem of antimicrobial resistance (AMR). AMR occurs when microbes evolve the ability to survive the exposure to antimicrobial agents that were previously effective against them. Although resistance is a natural evolutionary process, its pace and scale have been drastically accelerated by human activity. The inappropriate and excessive use of antibiotics in both human and animal health, including their unregulated prescriptions, incomplete courses, and routine use in livestock, has created intense selection pressure, driving the emergence and spread of resistant strains especially those with plasmid-borne resistance genes (2).

As a result, AMR is becoming a serious global health threat with wide-ranging implications. Infections that were once easily treatable are now harder, and sometimes impossible to manage, leading to longer illnesses, higher medical costs, and greater risk of complications or deaths (3). In 2019, AMR was associated with 4.95 million deaths globally, including 1.27 million directly caused by drug-resistant infections. Forecasts suggest that without urgent intervention, annual deaths could reach up to 10 million annually by 2050 (4).

Beyond, morbidity and mortality, AMR also threatens to undermine the health systems and economies. Resistant infections lead to prolonged hospital stays, delay in treatment success, and places a significant financial burden on individuals and governments. According to the World Bank, the global healthcare cost associated with AMR could rise to US$ 1 trillion by 2050. The economic impact extends beyond the health sector, posing a serious threat to global stability. Simulations suggests that if left unchecked, AMR could reduce the annual global gross domestic product (GDP) to levels similar to those seen in the 2008-2009 financial crisis, but with effects lasting for decades. Low-income countries are expected to bear the greatest burden with millions potentially pushed to extreme poverty, making it even harder for countries to meet global development goals. As resistant infections rise across both high- and low-resource settings, AMR continues to act as a silent pandemic with growing social, economic, and health consequences (2,5).

Mechanisms of Resistance

To better understand how AMR develops and spreads, it is important to look at the underlying biological strategies that bacteria use to evade antimicrobial action.

Bacteria are known for their ability to adapt and thrive in a variety of settings, including the presence of antimicrobial agents. This adaptability is partly due to their genetic plasticity, which allows them to develop resistance by either acquiring genes from other microbes, or by going through mutations that provide survival advantage (6). While some bacteria are naturally resistant to certain drugs, many develop resistance through the exposure to antibiotics overtime. This resistance can then be passed on within or across bacterial populations.

Although different resistance mechanisms have evolved in bacteria, they can be broadly grouped into four main categories: limiting drug uptake, active drug efflux, drug target modification, and drug inactivation (7). The specific mechanism used often depend on the bacterial species, the type of drugs involved and the surrounding environmental conditions. Generally, Gram-negative bacteria are known to use all four of these strategies while, Gram-positive bacteria mainly use drug target modification and inactivation (8).

While all resistance mechanisms play important roles, drug inactivation is particularly concerning due to its broad impact, serious clinical consequences, and resistance to countermeasures. A key reason for this concern is the prevalence of beta-lactamase production, where enzymes deactivate beta-lactam antibiotics. These enzymes have become a major focus in the fight against AMR, especially with the increasing emergence of extended-spectrum beta-lactamases (ESBLs) and carbapenemases.

Beta-Lactams and Beta-Lactamases

Beta-Lactam antibiotics are among the most widely used and effective drugs for preventing and treating bacterial infection. They are considered first-line treatments for critically ill patients that are suffering from septic shock or sepsis (9). Defined by their characteristic beta-lactam ring, these antibiotics target penicillin-binding proteins (PBPs), which are essential for bacterial cell wall synthesis. By disrupting this process, they weaken the cell wall, causing the bacteria to rupture under osmotic pressure, and ultimately lead to cell death (10).

The major classes of clinically used beta-lactam antibiotics include penicillins, cephalosporins, carbapenems, monobactams, and carbenicillin. These classes vary in their effectiveness against different bacterial pathogens and in their susceptibility to degradation by beta-lactamase enzymes (11).

Beta-lactamases are a diverse group of enzymes produced by bacteria and are a major cause of resistance to beta-lactam antibiotics (10). They function by breaking down the beta-lactam ring, rendering the antibiotic ineffective. This resistance mechanism is the most common form found in clinically important Gram-negative bacteria. The presence and type of beta-lactamase produced by a bacterial strain can significantly influence which antibiotics remain effective (12).

In recent decades, the prevalence of beta-lactamases has significantly increased. This trend has paralleled the widespread and often inappropriate use of antimicrobial agents, suggesting a strong link between antibiotic prescribing patterns and the evolution and spread of resistance.

Two of the most clinically significant beta-lactamases are ESBLs and carbapenemases. ESBLs confer resistance to a wide range of penicillins and cephalosporins, including third and fourth generation cephalosporins, which were once considered reliable treatments for serious Gram-negative infections (10,11). The most prevalent and clinically relevant ESBL genes include *blaTEM*, *blaSHV* (sulfhydryl variable), and *blaCTX-M* (cefotaxime-hydrolyzing beta-lactamase), which are commonly transmitted through horizontal gene transfer within the *Enterobacterales* family. In some cases, the activity of ESBLs can be inhibited by beta-lactamase inhibitors such as clavulanic acid, tazobactam, or avibactam, which are combined with beta-lactam antibiotics to restore their efficacy (13).

Carbapenemases, on the other hand, are capable of hydrolyzing carbapenems, which are the last-resort antibiotics for treating multidrug-resistant (MDR) infections. Well-characterized carbapenemase genes include *Klebsiella pnaumoniae carbapenemase (blaKPC*), *New Delhi metallo-β-lactamase (blaNDM)*, and *oxacillinase-48 (blaOXA-48)-like* enzymes, all of which contribute to the growing challenge of carbapenem resistance. Unlike many ESBLs, carbapenemases are typically not inhibited by traditional beta-lactamase inhibitors, making infections caused by carbapenemase-producing organisms especially difficult to treat (14). The increasing prevalence of these enzymes in multi-drug-resistant gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pnaumoniae*, and *Pseudomonas aeruginosa*, presents a serious global health challenge by limiting treatment options, increasing the risk of pan-resistance, and complicating infection control efforts.

Global Priority Beta-Lactamase-Producing Pathogens

The increasing prevalence of beta-lactamase-producing pathogens has become a major global concern. The scale of this problem has been highlighted by the World Health Organization (WHO) in its 2024 Bacterial Priority Pathogen List (BPPL), which serves as a strategic tool to guide research, development, and policy responses to AMR. It categorizes 15 families of

resistant bacteria into critical, high, and medium priority levels based on public health impact, transmissibility, treatability, and the availability of treatment options. It aims to focus global efforts and resources towards pathogens posing the most urgent resistance threats (15).

In the 2024 edition, carbapenem-resistant *Enterobacterales* and third-generation cephalosporin-resistant *Enterobacterales* are designated as critical priority pathogens. This classification reflects their increasing resistance, rapid global spread, and the lack of effective treatment options, especially in low-resource settings.

Klebsiella pneumoniae as a Key Resistance Driver

Enterobacterales are a large order of gram-negative bacteria comprising over 250 species, which collectively include the most common human pathogens (16). Among them, *Klebsiella pneumoniae* has emerged as a particularly significant contributor to the global spread of AMR.

K. pneumoniae is a commensal bacterium that naturally colonizes the gastrointestinal tract and nasopharynx of healthy individuals, as well as a variety of environmental reservoirs such as water, soil, and plants (17). Despite being naturally present, *K. pneumoniae* has become a major opportunistic pathogen, causing both hospital- and community-acquired infections, including pneumonia, sepsis, urinary tract infections, meningitis, and wound infections. Hypervirulent strains can also cause liver abscesses and endophthalmitis, which may progress to bloodstream infections. It is now a leading cause of healthcare-associated infections, particularly in intensive care units and among vulnerable populations, including neonates in Africa and Asia (18).

Its role in AMR has earned *K. pneumoniae* a place among the “ESKAPE” pathogens, a group of bacteria known for their capacity to “escape” the effects of most clinically used antibiotics (19). *K. pneumoniae* not only exhibits intrinsic resistance to penicillins through chromosomally encoded enzymes like *SHV-1* but also readily acquires additional resistance genes, including those encoding ESBLs and carbapenemases (20). The widespread overuse of broad-spectrum antibiotics, particularly carbapenems, has further driven the emergence of carbapenem-resistant Enterobacteriaceae (CRE), with *K. pneumoniae* being one of the most prevalent and concerning species (21).

Additionally, recent evidence shows that some hypervirulent lineages, once typically antibiotic-susceptible, are now acquiring resistance traits. This convergence of hypervirulence and multidrug resistance further limits treatment options and increase the risk of severe, untreatable infections (22).

A key factor underlying *K. pneumoniae*'s success as a resistance driver is its strong association with plasmid-mediated gene exchange (23). Genomic studies estimate that most (67.5%) acquired AMR genes in clinical *K. pneumoniae* are plasmid-borne, facilitating rapid horizontal gene transfer both within and across species. These plasmids not only boost the bacterium's ability to accumulate multiple resistance determinants but also contribute to the global dissemination of high-risk clones, especially in regions with high antibiotic pressure. As a result, *K. pneumoniae* acts as an important reservoir and driver of AMR spread worldwide (20).

Plasmids

Bacteria evolve quickly because of two main reasons: their huge population size and through horizontal gene transfer (HGT). HGT in prokaryotes refers to the transfer of genetic material between individual bacteria rather than by vertical inheritance from parents to offsprings (24). This process enables bacteria to rapidly acquire new traits such as antibiotic resistance through mechanisms including transformation (uptake of free DNA), transduction (virus-mediated transfer), and conjugation (direct cell-to cell DNA transfer) (25).

One of the most common vehicles for HGT in bacteria are plasmids. Plasmids are small, circular DNA molecules that replicate independently of the bacterial chromosome and often carry genes that can benefit their hosts. They play a major role in bacterial evolution because of their ability to move between cells and transfer beneficial traits within and between bacterial species. While they can sometimes be seen as selfish or parasitic genetic elements due to the potential burden they impose on their hosts, they often carry traits that can increase the fitness of their host under extreme stress. This dual nature of plasmids make them powerful drivers of evolution, particularly in the spread of AMR (26).

Their ability to transfer between bacterial cells is largely determined by the presence or absence of specific mobility genes. Conjugative plasmids (pCONJ) possess the full set of machinery required for autonomous DNA transfer, including a relaxase enzyme, a Type IV Secretion System (T4SS), and mating pair formation (MPF) components. In contrast, decayed conjugative plasmids (pdCONJ) retain only some of these elements (typically a relaxase and partial MPF genes) but are no longer capable of self-transfer. Mobilizable plasmids (pMOB) encode a relaxase and an origin of transfer (oriT), allowing them to be mobilized by the conjugation systems of other plasmids. Finally, pMOBless plasmids lack relaxase genes altogether, and their ability to transfer is generally unknown or considered absent. This capacity for DNA movement across bacterial populations and even distantly related species makes plasmids powerful agents of HGT. Whether autonomous (via conjugative plasmids) or

mobilized (via mobilizable plasmids), conjugation is a primary mechanism for HGT, facilitating the spread of crucial adaptive traits, including antibiotic resistance genes (ARGs) (27).

Plasmids also contain essential features that ensure their replication and stable inheritance within bacterial hosts. Each plasmids carries a replicon, which includes the origin of replication (oriV), a specific initiation site for replication, and the associated genes needed to initiate and control this process. Plasmids use different replication strategies, often depending on their size and stability requirements. Additionally, plasmids are grouped into incompatibility (Inc) groups based on their replication and inheritance systems to where plasmids from the same group typically cannot coexist in a single cell due to interference with each other's maintenance. Together, these replication and stability features enable plasmids to persist and spread across diverse bacterial populations (28).

Beyond basic functions, plasmids are mobile genetic elements that often carry accessory genes, such as ARGs, virulence factors, or metabolic enzymes, that can confer advantages to their bacterial hosts under certain conditions. Through recombination, they can rapidly acquire and rearrange genetic material, making them highly variable and adaptable entities (27,28).

To better understand plasmid diversity and evolution, several classification systems have been developed. The earliest were based on observable traits, such as fertility or resistance (e.g. F-factors, R-factors), but were later replaced by the incompatibility grouping, which became the basis for plasmid classification (29). While Inc typing remains relevant, particularly for tracking AMR, its limitations, including phenotypic ambiguity and difficulty in interpretation, have led to the development of newer, genome-based methods. One product of these method is the concept of Plasmid Taxonomic Units (PTUs) which were developed aiming to address the limitations of earlier methods. PTUs group plasmids with shared genetic backbones using whole-genome similarity, offering a more biologically meaningful framework similar to bacterial species (30). However, even whole-genome methods face challenges due to the complex nature of plasmids, limitations in database coverage, and variability in sequence conservation within groups. No current ia .

Plasmid Types as Vehicles of AMR Spread

The link between plasmid types and AMR has been widely documented across both clinical and environmental settings. Numerous studies have shown that certain plasmid types, particularly those identified by Inc grouping or more recently by PTUs, have been repeatedly associated with the carriage and dissemination of clinically important resistance determinants, including ESBLs and carbapenemases (31,32). Several plasmid types have also been linked

to the carriage of key resistance genes in *K. pneumoniae*, and that certain plasmids commonly co-occur with both resistance and virulence traits, especially in hypervirulent strains (33,34).

Beyond species-specific associations, broader surveys of plasmid genomes have shown that AMR genes tend to cluster within certain plasmid types, particularly those capable of coexisting within the same bacterial host. These plasmids create an environment that promotes gene sharing and recombination, accelerating the spread of multidrug resistance (35).

The Present Study

Despite the growing understanding of AMR drivers globally, our understanding of how specific plasmid types contribute to resistance in *K. pneumoniae* remains incomplete, particularly in settings with limited surveillance infrastructure or access to high- resolution genomic typing. This knowledge gap is especially concerning given *K. pneumoniae*'s role as a reservoir of both resistance and, potentially, virulence. There is also strong evidence linking particular plasmid types to the carriage of ESBLs, carbapenemases, and other clinically important resistance genes in isolates from healthcare settings.

While existing classification systems have helped reveal broad trends in plasmid-AMR associations, many still lack detail, standardization, or interpretability. To address these limitations, this study makes use of an in-house plasmid typing framework, designed to robustly cluster plasmids based on genomic similarity and track their distribution across bacterial hosts and resistance contexts.

By integrating plasmid-level data with resistance gene profiles, this work aims to identify specific plasmid types associated with beta-lactamase carriage in *K. pneumoniae*. In doing so, it seeks to contribute to a deeper understanding of AMR dynamics and highlights plasmid types as potential targets for molecular surveillance, especially in clinical settings where resistance patterns are complex and rapidly evolving.

RESEARCH OBJECTIVES

This study investigates the role of plasmids in the dissemination of beta-lactamase genes in *Klebsiella pneumoniae*. Specifically, it aims to:

1. Describe the distribution of antibiotic resistance genes specifically beta-lactamase genes, and plasmid lineages across *K. pneumoniae* genomes.

2. Identify plasmid types associated with beta-lactamase gene carriage.
3. Explore whether specific plasmid types show geographic structuring that may contribute to the regional spread of beta-lactam resistance.

Specific Objectives:

To achieve the above aims, this study specifically sought to:

a. Describe the diversity and structural features of plasmids carrying beta-lactamase genes in a global collection of *K. pneumoniae* genomes.
b. Test the associations between plasmid types and beta-lactamase gene carriage.
c. Evaluate whether the association between plasmid type and beta-lactamase carriage differs across continents.
d. Conduct sub-analyses focused on extended-spectrum beta-lactamase (ESBL) and Carbapenemase gene carriage as the two major beta-lactamase classes of clinical relevance.
e. Highlight plasmid types most relevant to resistance spread in different continents.

METHODS

Data

The primary data used for this study consists of all available complete, high-quality *Klebsiella pneumoniae* genome assemblies downloaded from the NCBI Refseq (Reference Sequence) database, along with associated metadata retrieved from GenBank. Refseq is a a curated non-redundant collection of genomic sequences and associated annotations that is maintained by the National Center for Biotechnology Information (NCBI) and it is publicly available at: https://www.ncbi.nlm.nih.gov/refseq/ (36).

A total of 2,190 K. genomes were initially retrieved from Refseq. To further ensure quality and reduce redundancy, genome filtering was performed using PanACoTA (PANgenome with Annotations, COre identification, Tree and corresponding Alignments), a software that provides tools to compare genomes of bacterial species to study their genetic similarities, and evolutionary relationships (37). Filtering was based on a minimum Mash (a bioinformatics tool used to estimate genetic distance between genomes) distance threshold of 1×10^{-10}. After filtering, 2,074 genomes were retained and used for all subsequent analyses, including ARG detection, plasmid typing, and statistical modelling.

Metadata associated with these genomes was obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/), including geolocation (country and in some cases city), collection date, isolation source, and host. Upon inspection, only the geolocation and collection dates were usable for analysis (38). The other fields contained a lot of missing information or inconsistent reporting formats, limiting their interpretability.

For the geolocation, only the country was extracted and used for geographic grouping. City-level information was excluded as it was inconsistently reported. In total, the dataset included genomes from 59 different countries. To improve interpretability and maintain statistical power, countries were grouped by continent, which was used in the analysis.

For the collection date, only the year of isolation was retained. In cases where multiple years or specific dates were listed, the oldest available date was selected. Although not used in the current analysis, this variable is preserved for potential use in the future temporal analysis.

Feature Detection

To characterize genomic features of *K. pneumoniae*, two tools were used: Kleborate and AMRFinderPlus. These tools allowed for species confirmation, genotyping, and ARG detection across the collected *K. pneumoniae* genomes.

- **Kleborate**

Kleborate is a genotyping tool for the *Klebsiella pneumoniae* species complex (KpSC), commonly used to identify clinically important lineages. In this study, Kleborate v3 was run using the --preset kpsc module to confirm that all genomes were indeed *K. pneumoniae* and to determine their multilocus sequence types (MLST) based on the seven-locus Pasteur scheme, which classifies isolates by comparing sequences at seven housekeeping genes (39,40). Sequence type (ST) information was not included in the main analysis but is available in the supplementary results.

- **AMRFinderPlus**

AMRFinderPlus is a tool developed by NCBI that detects AMR genes as well as virulence, stress response, and mutation related genes (41). The tool was run on the protein FASTA files for each *K. pneumoniae* genome. The resulting outputs were used to explore the distribution of ARGs, their resistance class, and genomic locations (plasmid or chromosome). Beta-lactamase genes were later selected for focused analysis. These genes were grouped into families based on gene name and known function. Binary variables were then created to

indicate the presence or absence of beta-lactamase genes, as well as specific subtypes for ESBLs and carbapenemases, to use in downstream analysis.

Plasmid Identification and Typing

Plasmid-level information was obtained using an in-house typing framework developed within the research group. This classification system groups plasmids based on known genetic markers and similarity thresholds, and defines plasmid types independently of the commonly used incompatibility (Inc) and PTU systems which are the standard grouping systems currently used in plasmid classification. Each plasmid in the dataset was assigned a numerical plasmid type, along with associated features including its size (in base pairs), mobility, and replicon type. Plasmid size was examined during exploratory analysis to assess its distribution across the dataset but was not included in downstream statistical modeling. Plasmid type served as the central predictor in the analyses aimed at identifying associations with beta-lactamase gene carriage.

Exploratory Analysis

Descriptive analysis was performed at both the genome and plasmid levels to characterize the distribution of ARGs, plasmid features and typing results across the dataset. Visualizations included frequency distributions, bar plots, and summary tables. These steps provided a descriptive overview of the dataset and highlighting key variables for further analysis.

Statistical Analysis

All statistical analyses were conducted in R version 4.5.0 (2025-04-11) to explore the associations between plasmid types and beta-lactamase carriage across *K. pneumoniae* genomes. The primary aim was to identify patterns in the distribution of resistance-related features and assess whether certain plasmid types were associated with the presence of beta-lactamase genes, particularly ESBL and carbapenemase-encoding genes, and whether these association varied by geographic region.

Findings from the association analyses guided the development of regression models, which were used to complement residual-based results. These models helped quantify the direction and relative strength of the observed associations. Given the sparse and uneven nature of the dataset, model outputs are interpreted with caution. Rather than providing precise effect estimates, the models serve to support overall trends and assess the robustness of associations across different contexts.

- **Association Analysis**

To account for the large and sparse dataset, chi-squared tests with Monte Carlo simulated p-values were used to assess associations between plasmid types and the presence of beta-lactamase genes, including within resistance subgroups (ESBL and carbapenemase) to identify disproportionately represented plasmid types. The same approach was applied to test associations with continent of isolation to explore geographic structuring. Standardized residuals were then visualized to highlight plasmid types enriched in specific regions and the results of the residuals formed the core of the exploratory findings and the primary analytical output of this study.

- **Modelling**

As a complementary analysis to the residual-based association tests, logistic regression models were used to further examine the relationship between plasmid types and beta-lactamase gene carriage. The outcome variable was defined as presence (1) or absence (0) of any beta-lactamase gene, with plasmid type is the main predictor to assess whether certain plasmids are more likely to carry these genes.

Three models were constructed for the analysis: a crude (unadjusted) model, an additive model that included continent of isolation as a covariate, and an interaction model that incorporated a plasmid type-by-continent term to test for effect modification.

The effect of continent alone on the outcome was also tested as an evaluation of the two possible predictors before combining them in a model. It served mainly as a comparison for understanding possibilities of confounding and building up the understanding of the additive and interaction models.

This modelling strategy was then applied in subgroup analyses, where separate models were built for ESBL and carbapenemase outcomes (both binary), each following the same three-model framework.

To examine the regional patterns more closely, additional stratified analyses were conducted for plasmids isolated from Asia and Europe, which were the two continents with the strongest signals in exploratory analysis. In these models, plasmid type remained the primary predictor without additional covariates.

Reference categories for the models were selected based on interpretability and relevance to each context. In the general and subgroup models, “Other” and “Asia” were used as the reference categories for plasmid type and continent, respectively, as they had the highest

number of observations. In the Asia-stratified models, plasmid type “486,” a dominant local plasmid in the region, was chosen as the reference. A sensitivity analysis using plasmid type “333,” which is a carbapenemase-carrying plasmid specific to Asia, was also performed, however, due to its low frequency and resulting model instability, “486” was retained as the reference. For the Europe-stratified models, plasmid type “276” was selected based on its strong observed association with carbapenemase gene carriage in the exploratory analysis. A sensitivity analysis using “Other” as the reference further supported this choice.

- **Model Fit and Evaluation**

Model fit was assessed using Akaike Information Criterion (AIC) and log-likelihood comparisons. Interaction terms were retained only when they significantly improved model fit.

- **Robust Standard Errors**

To account for the fact that multiple plasmids can come from the same genome, a mixed-effects logistic regression model was initially considered. This model included a random intercept for genome to capture any genome-level variability that might affect beta-lactamase gene carriage. The intraclass correlation coefficient (ICC) was calculated to estimate how much of the variation was due to the differences between genomes (see Supplementary Information). However, given the minimal contribution of genome-level clustering, multilevel modelling was not pursued further. Instead, robust standard errors were used in all the models to account for possible model misspecification, uneven group sizes, and variability in the outcome. Because some plasmid types were much more frequent than others, and the outcome distribution may have varied across groups, the use of robust standard errors provided more reliable and stable estimates.

Computational Environment

Data processing, analysis, and tool execution were carried out on a Linux-based system using a combination of command-line tools, custom scripts, and Python. Tasks such as genome management, AMR and other feature detection, and dataset merging were performed using basic shell commands, Python scripts, and R (v4.5.0) for statistical analysis and visualization.

Job submissions for resource-intensive tasks were managed through SLURM on the LBBE/PRABI computing cluster.

RESULTS

AMR Distribution

A total of 351 unique ARGs were identified across 2,074 *K. pneumoniae* genomes, spanning 20 different drug classes. Of the 29,778 ARG observations identified in the core subset of AMRFinderplus results, over half (55.93%) belonged to three drug classes: beta-lactams (27.43%), aminoglycosides (16.88%), and phenicol/ quinolone (11.62%). A full table summarizing drug classes, total unique genes, and gene observations across the full dataset is included in the Supplementary Results (Supplementary Table S1).

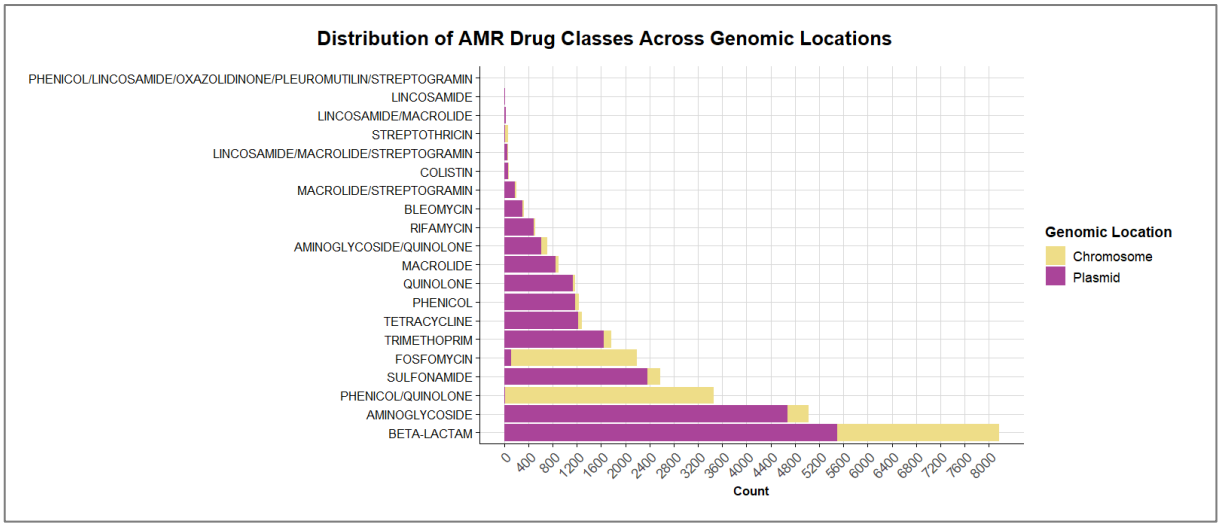

Figure 1: Distribution of antimicrobial resistance (AMR) gene classes by genomic location (chromosome vs. plasmid).
The bar chart shows the counts of AMR genes grouped by drug class and their genomic location.

Most of the ARGs were located on the plasmids (68.71%) with the remaining found on the chromosome (31.29%). For most drug classes, plasmid carriage of ARGs was more common than chromosomal integration. However, Fosfomycin and phenicol/ quinolone resistance genes were notable exceptions as they were almost exclusively found in the chromosome (Figure 1).

- **Plasmid- Associated AMR Genes**

Out of the 351 unique ARGs identified in the full dataset 286 genes were detected in the plasmid-associated contigs.

As with the full dataset, the beta-lactam class showed the highest gene diversity (126 gene variants) and accounted for the highest proportion of observations (26.86%) among the 20,459 total plasmid-borne ARGs. This was followed by aminoglycosides and sulfonamides. Notably, sulfonamide resistance genes were more common in plasmids than phenicol/ quinolone genes which were previously ranked third in the overall ARG distribution. This shift reflects differences

in how specific resistance classes are distributed between the chromosome and plasmid compartments. A table summarizing plasmid-borne ARGs by drug class is provided in the Supplementary Results (Supplementary Table S2).

- **Beta-Lactamase Gene Family**

Given its high gene diversity and clinical importance, beta-lactamase genes were selected for focused analysis. This resistance class accounted for the largest number of unique ARGs identified and included several of the most frequently observed resistance determinants across the dataset.

In total, 21 beta-lactamase gene families were identified, with *blaSHV*, *blaCTX-M*, *blaTEM*, *blaOXA*, *blaKPC*, and *blaNDM* being the most prevalent. Together, these six families represented approximately 89.15% of all beta-lactamase genes identified in the full dataset and 90.31% of those found on plasmids. To provide functional context, the gene families were further grouped according to their associated resistance genotypes. Specifically, *blaCTX-M*, *blaSHV*, and *blaTEM* were categorized ESBLs, while *blaKPC*, *blaNDM*, *blaOXA*, *blaVIM*, and *blaIMP* were classified as carbapenemases (Figure 2).

To support downstream analyses, binary variables were created to indicate the presence or absence of any beta-lactamase gene, as well as specific groupings for ESBL and carbaenemase gene based on the family classification above.

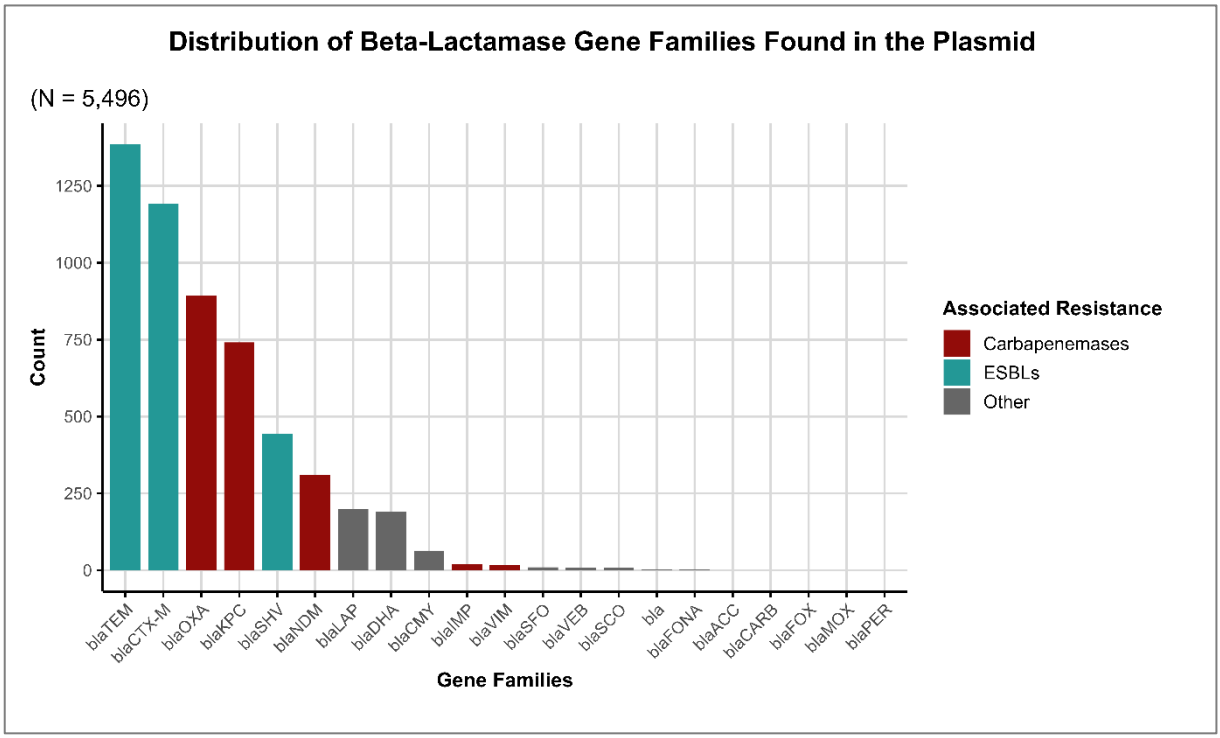

Figure2: Distribution of β-Lactamase gene families found in the plasmid.
The bar chart shows the counts of β-lactamase genes found in the plasmids, grouped by gene family, and categorized by associated resistance genotype.

Plasmid Distribution

- **General Plasmid Landscape**

Out of the 2.074 *K. pneumoniae* genomes analyzed, 1,959 (94.50%) had at least one plasmid and among these plasmid-positive genomes, the majority (1,740; 88.80%) carried at least one plasmid associated with AMR.

Across the full dataset, a total of 7,147 plasmids were identified, with genomes typically carrying between two to three plasmids each. Of these, 3,795 plasmids (53.10%) were classified as AMR plasmids, based on the presence of at least one AMR gene.

A substantial proportion of AMR plasmids (2,827; 39.65% of all plasmids) carried at least one beta-lactamase gene. Within this subset, 36.68% carried both ESBL and carbapenemase genes, 28.19% carried only ESBL genes and 24.90% carried only carbapenemases (Table 1). These groupings were used to define subgroups for downstream chi-square and regression analyses, with plasmids carrying only ESBL or only carbapenemase genes examined separately to evaluate their associations with plasmid type and other predictors.

Table 1. Distribution of β-Lactamase–Positive Plasmids by ESBL and Carbapenemase Gene Presence

ESBL	Carbapenemase	n	Percent (%)
0	0	289	10.22
0	1	704	24.90
1	0	797	28.19
1	1	1037	36.68

- **Structural and Functional characteristics**

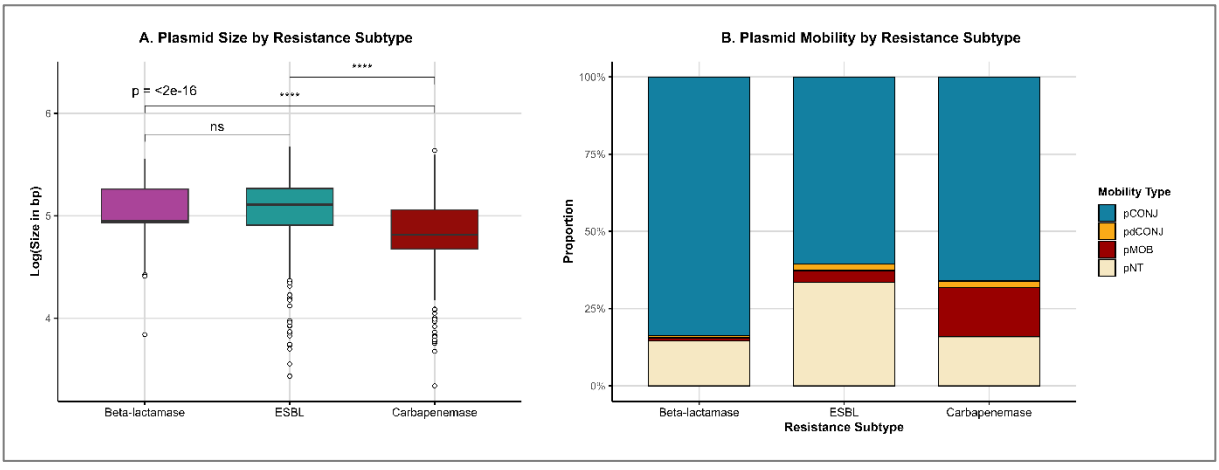

Figure 3: Structural and functional characteristics of β-lactamase, ESBL, and carbapenemase-carrying plasmids
(A) Log-transformed plasmid size distributions by resistance subtype, with overall significance assessed using the Kruskal-Wallis test and pairwise comparisons using Wilcoxon rank-sum tests.
(B) Distribution of plasmid mobility types show a predominance of conjugative plasmids across all subtypes.

Plasmid sizes across the dataset varied widely. Beta-lactamase-carrying plasmids tended to be larger on average than the full plasmid set, with a median size of 112 kb compared to 77 kb in the general dataset. ESBL-only plasmids had the largest median size (121 kb), while carbapenemase-only plasmids were generally smaller, with a median of 65 kb.

In terms of mobility, most plasmids carrying beta-lactamase genes were conjugative (64.24%), followed by non-transmissible (27.24%). This pattern was consistent across subgroups, with both ESBL-only and carbapenemase only plasmids showing high proportions of conjugative mobility (64.87% and 66.05% respectively).

- **Plasmid Types**

Using the in-house typing framework, a total of 224 plasmid types were identified across the full dataset. Among these, 108 types were found in plasmids carrying ARGs, and 95 types were identified specifically among plasmids carrying at least one beta-lactamase gene. Within the beta-lactamse positive plasmids, 16 types always carried only ESBL genes (e.g. 23, 4420 and 61), 8 always carried only carbapenemase genes (e.g. 333), and 6 types always carried both groups together (e.g. 324 and 482). The remaining 62 types were shared types (e.g. 486, 276, 68 and 94), appearing in multiple beta-lactamase subgroup combinations, indicating the same plasmid types may carry different resistance genotypes across genomes.

Although the dataset included a wide variety of plasmid types, the majority were rare (less than 1% of total plasmid observations). To focus the statistical analysis on plasmid types with broader relevance, the top 20 most frequent plasmid types were selected as candidate predictors. Each of these types represented at least 1% of all typed plasmids, excluding unclassified types. This selection captured a biologically meaningful range of plasmids, including those frequently associated with beta-lactamase genes, those carrying other AMR genes, and some without any known resistance genes (Figure 4.A). This heterogeneity supports their use as predictors, as they allow comparisons across plasmids with differing resistance profiles and structural backgrounds.

When focusing specifically on plasmids that carry beta-lactamase genes, the top 20 plasmid types accounted for the majority of beta-lactamase-positive plasmids. Many of these types showed high proportions of AMR gene carriage, including some with near-complete association with beta-lactamase genes (Figure 4.B). These frequently occurring beta-lactamase plasmid types served as an informative reference when examining resistance patterns and were used descriptively to guide further subgroup comparisons in both exploratory and regression analyses.

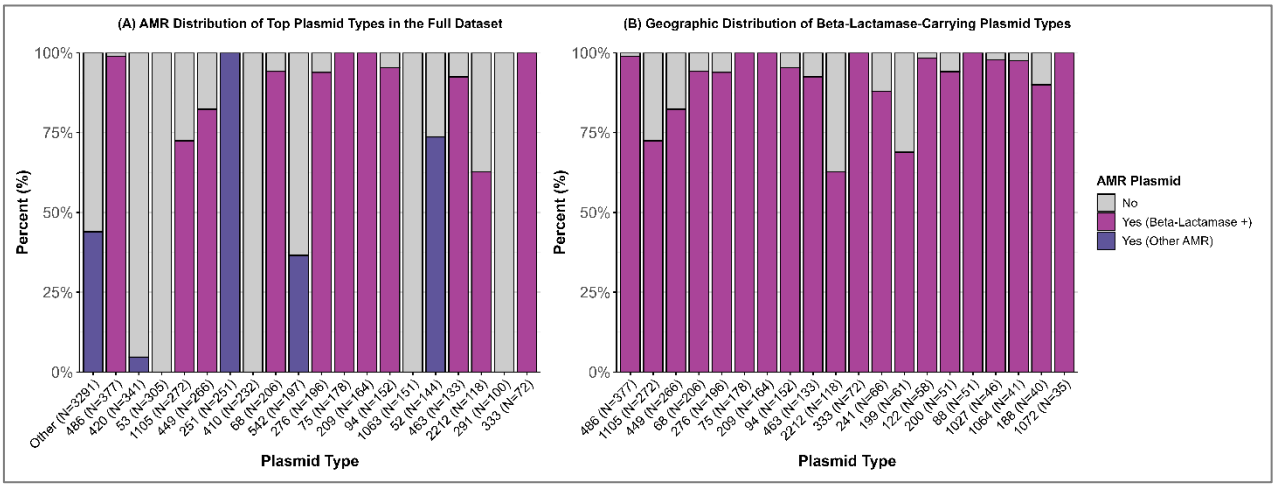

Figure 4: AMR distribution across common plasmid types in the dataset

(A) Plasmid types representing at least 1% of all plasmids in the entire dataset.

(B) Top 20 plasmid types associated with β-lactamase carriage, each also present at ≥1% frequency.

Bar segments indicate the proportion of plasmids carrying AMR genes, with specific highlighting of those carrying beta-lactamase genes or other ARGs.

Geographic Distribution

Plasmids included in this study were isolated from 59 countries across six continents, along with a portion from unknown or unidentified origins. Country-level distributions deduced from the original metadata were highly skewed, with China accounting for over a third of all the plasmids (37.48%), followed by the United States (9.57%) and Taiwan (5.83%). Other countries contributed fewer than 5% each and most having only less than 1% each. To account for this skew in the distribution and to facilitate broader comparisons for succeeding analyses, country data were grouped by continent. Asia was the most represented region (54.86%), followed by Europe (21.24%) and North America (10.45%). Africa, South America, and Oceania each contributed fewer than 5% of all the plasmid (Figure 5). This continent level-classification was u sed in both exploratory and statistical analyses.

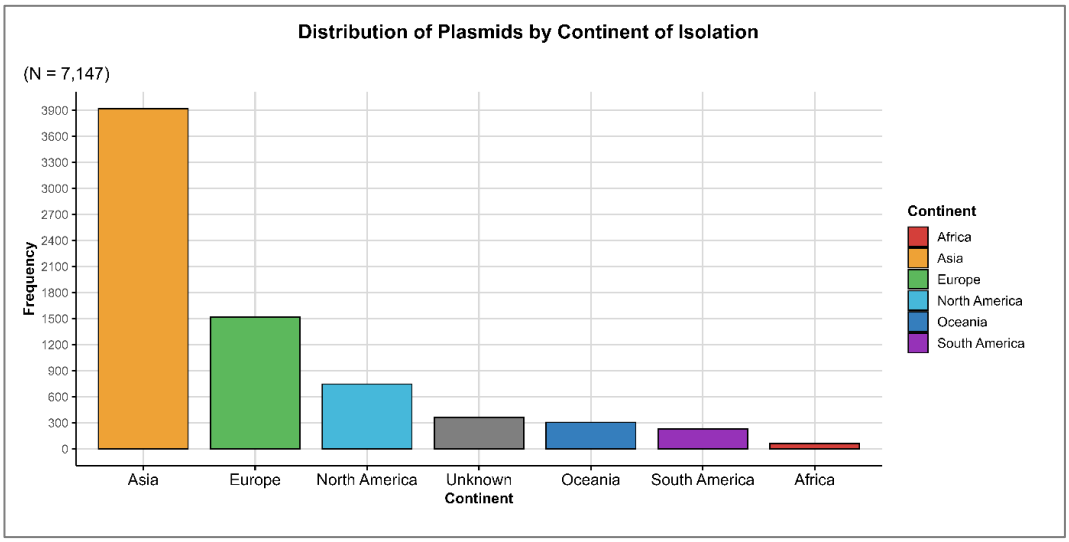

Figure 5: Distribution of plasmids by continent of isolation.

The distribution of plasmid types was examined to assess regional variability. The top 20 plasmid types in the full dataset exhibited distinct geographic patterns. Few types (486, 420, 53 and 410) were predominantly found in Asia, while others showed broader representation across Europe, North America, and other continents (Figure 6.A).

A similar pattern was observed when focusing on plasmids carrying beta-lactamase genes. While most beta-lactamase-associated types were widely distributed, a few were strongly associated with isolates from Asia (Figure 6.B). This variation supports the consideration of geography as a contextual factor in modeling and provides preliminary evidence for region-specific trends in plasmid-mediated resistance.

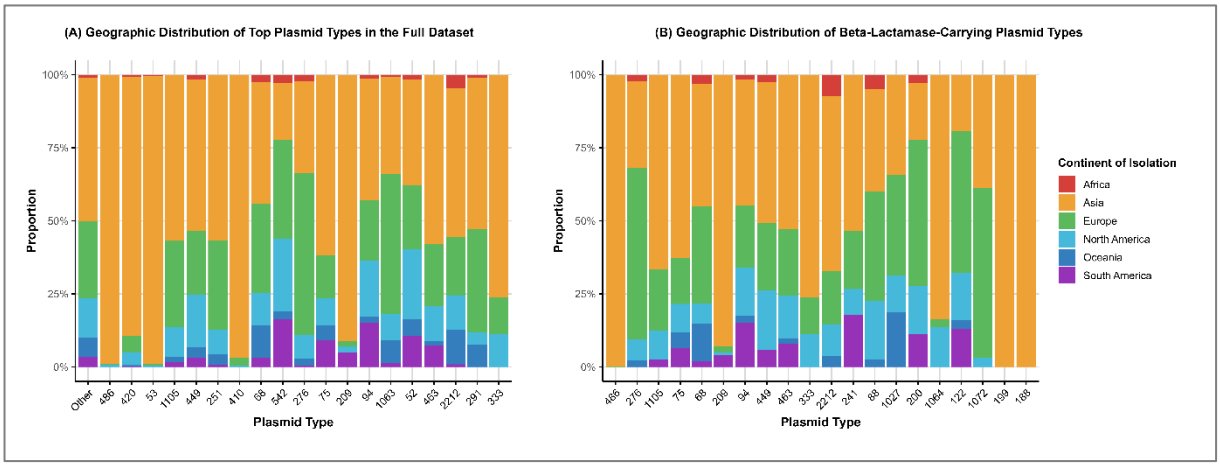

Figure 6: Geographic distribution of common plasmid types by continent of isolation.
(A) Top plasmid types in the full dataset, each representing at least 1% of total plasmids.
(B) Top plasmid types associated with β-lactamase carriage, each also occurring at ≥1% frequency.
Bars represent the proportional distribution of each plasmid type across six continents based on available metadata.

Statistical Associations

- **Plasmid Type and Beta-Lactamase Carriage**

Descriptive analysis showed that beta-lactamase genes were unevenly distributed across plasmid types, with some types showing high carriage rates while others had none. To formally assess whether plasmid type was associated with beta-lactamase gene carriage, chi-square tests were performed using the previously identified top 20 most frequent plasmid types in the dataset.

Because some expected counts were low, Monte Carlo simulation with 10,000 replicates was used to estimate p-values. Statistically significant associations were observed for all three resistance outcomes tested. Plasmid type was strongly associated with overall beta-lactamase gene presence ($X^2 = 2846.6$), ESBL-only carriage ($X^2 = 1986.8$), and carbapenemase-only carriage ($X^2 = 2023.5$), with simulated p-values < 0.0001 in all cases. These results reinforce

earlier observations of uneven resistance distribution and suggest that certain plasmid lineages may contribute more to the spread of specific resistance mechanisms.

To further explore the chi-squared associations, plasmid type distributions were examined separately within the ESBL-only and carbapenemase-only subsets (Figure 7). Although many plasmid types were shared across the two groups, including 276 and 94, their frequencies differed. For example, plasmid types 75 and 486 were the most frequent ones among ESBL-only plasmids, whereas plasmid types 276 and 333 were the most frequent ones among carbapenemase-only plasmids. Notably, plasmid type 333 was exclusive to the carbapenemase subset, while 276 appeared in both groups but was significantly more prevalent in the carbapenemase subset.

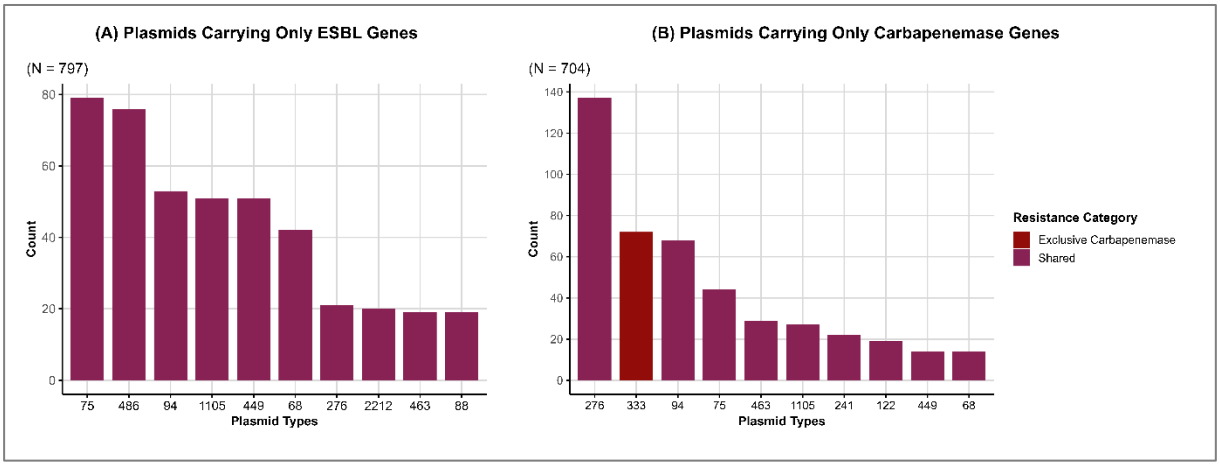

Figure 7: Top plasmid lineages carrying ESBL or carbapenemase genes.
(A) Top 10 plasmid types carrying only ESBL genes.
(B) Top 10 plasmid types carrying only carbapenemase genes.
Bar lengths represent lineage frequency within each group. Bar colors indicate whether plasmid types were exclusive to ESBL or carbapenemase genes, carried both, or were shared across resistance classes.

- **Plasmid Type and Continent of Isolation**

To investigate whether certain plasmid types were more prevalent in specific regions, chi-square tests were also conducted between continent of isolation and plasmid type using a subset of plasmids carrying beta-lactamase genes as well as both resistance subtypes (ESBL and carbapenemase). To reduce noise and ensure interpretability, only plasmid types that accounted for at least 1% of observations were included. The analysis excluded isolates from unknown regions, which comprised less than 0.5% of each subset and had negligible impact on the results.

The chi-squared test with Monte Carlo simulation (10,000 replicates) revealed a highly significant association between plasmid type and continent for beta-lactamase plasmids (X^2 = 921.2, p = 9.999e-05). Similar results were observed for the ESBL-only (X^2 = 438.5, p = 9.999e-

05) and carbapenemase-only subsets (X^2 = 276.5, p = 9.999e-05), indicating strong geographic structuring of plasmid lineages within each resistance group.
Residuals from these models were examined to identify the strongest contributors to these associations.

- **Analysis of Residuals**

Standardized residuals were examined to identify which continent and plasmid type combinations most strongly contributed t the observed association. Heatmaps showing the residuals highlighted distinct geographic patterns for several plasmid types across each resistance subset (beta-lactamase, ESBL only, and carbapenemase only) (Figures 8 - 10).

In the beta-lactamase dataset, the strongest enrichment was observed for plasmid type 486 in Asia (residual = 16.01), suggesting a disproportionately high presence of this lineage in the region and this same plasmid type is virtually absent from all other continents. Plasmid type 276, while also slightly enriched in Africa, showed significant enrichment in Europe (residual = 12.20). Type 68 was overrepresented in Oceania and moderately enriched in both Europe and Africa, indicating its association with beta-lactamase carriage across these three continents. Plasmid type 94 showed a moderate association with South America and a lesser but notable presence in North America. These patterns largely reflect the earlier distribution results.

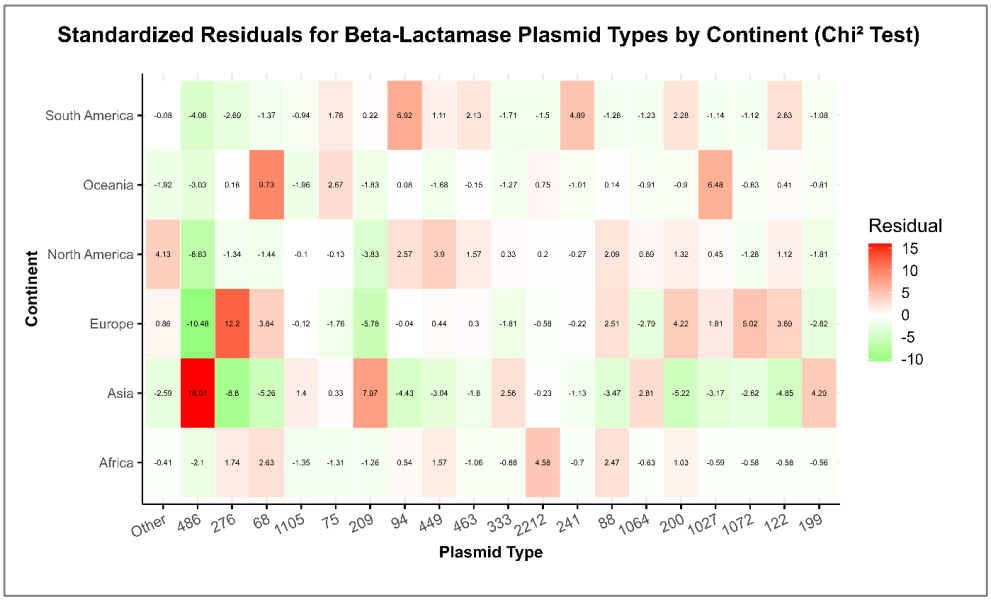

Figure 8: Standardized residuals for β-lactamase plasmid types by continent

The ESBL-only residuals offered further clarity on the residual patterns. Plasmid type 75, the most frequent ESBL-associated type, was enriched in both South America and Asia, with a smaller presence in Oceania. Plasmid type 486 was strongly associated with Asia, reinforcing its role as a potential ESBL carrier in the region. Interestingly, plasmid type 94 a which is a type

highly associated with both ESBL and carbapenemase gene carriage as shown by previous distribution analysis (third plasmid type in both subset), emerged as a highly represented ESBL type in both Europe and North America, but not in Asia. Meanwhile, plasmid type 68 is primarily associated with Oceania, while plasmid type 276 showed enrichment in Africa with a higher residual than in the general beta-lactamase carriage dataset, and North America, which was not visible in the broader beta-lactamase analysis.

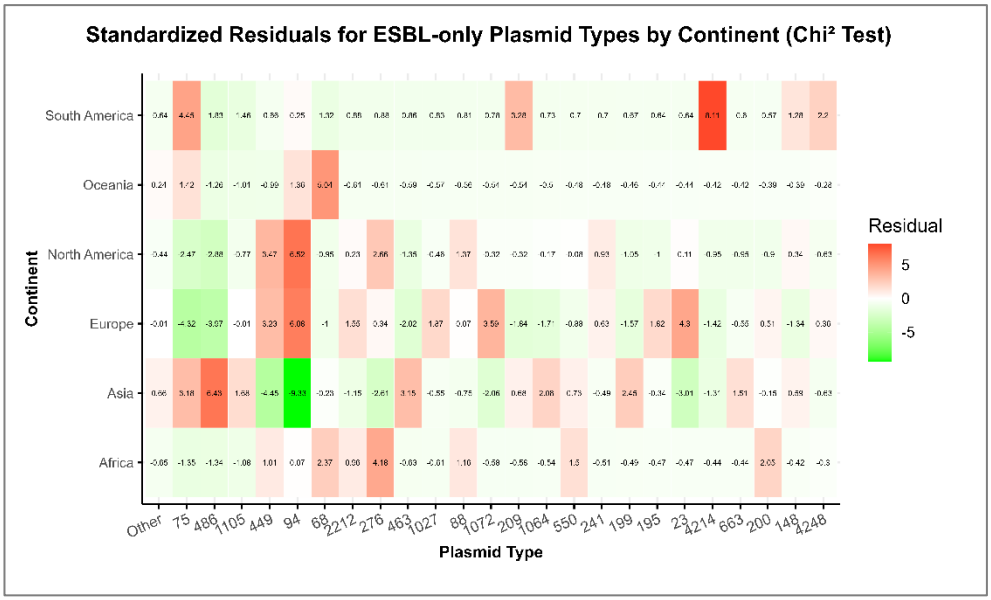

Figure 3: Standardized residuals for ESBL plasmid types by continent

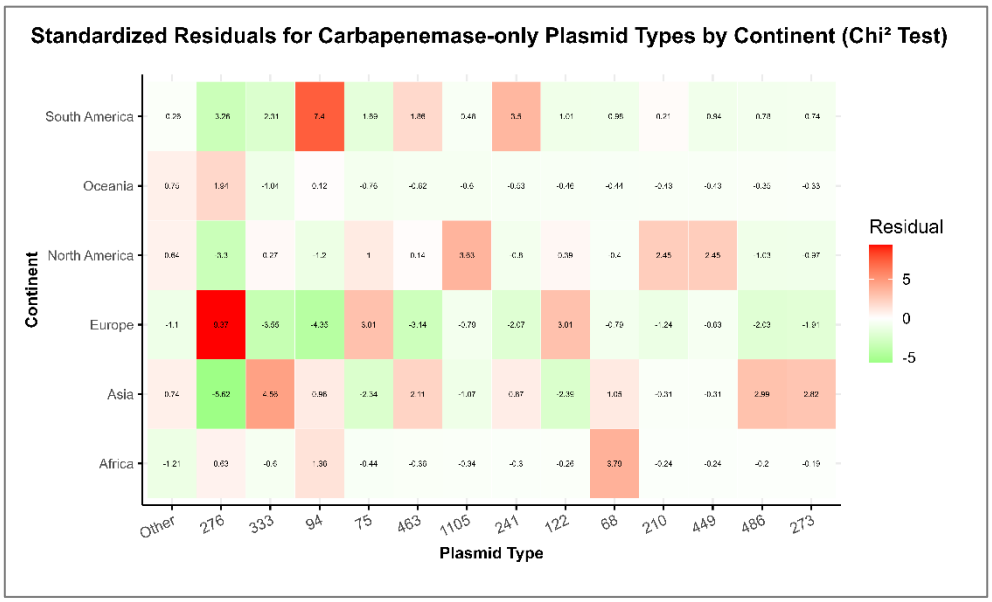

Figure 4: Standardized residuals for carbapenemase plasmid types by continent

In contrast, the carbapenemase-only residuals revealed a different set of associations. Plasmid type 276, while fund in ESBL contexts in other regions, was most strongly enriched in Europe when associated with carabapenemase gene carriage. Plasmid type 333 stood out for its

exclusive presence in Asia and notably always carried carbapenemase genes in the study. Type 94, a frequent carrier of both resistance groups, appeared strongly linked to South America for carbapenemase carriage. It also showed a smaller enrichment in Asia for carbapenemase plasmids, which is a pattern not seen in the broader beta-lactamase analysis and ESBL only subset. Plasmid 68, which had been highly associated with Oceania in the broader beta-lactamase and ESBL specific analyses, no longer showed any association with Oceania in the carbapenamse-only subset. Instead, it was more strongly associated with Africa and to a lesser degree with Asia. This shit suggests that plasmid type 68 in Oceania likely only carries ESBL genes in the context of beta-lactamase resistance.

Contextualizing Residual Patterns with Regression Modeling

To further explore the regional differences in plasmid–beta-lactamase associations identified through chi-squared tests and residual analysis, logistic regression models were constructed using beta-lactamase presence as the binary outcome and plasmid type as the main predictor. Continent of isolation was included to assess potential effect modification and examine whether geographic context influences the strength of these associations. These models were used as a complementary tool to support and contextualize patterns observed in the descriptive and residual analyses.

The modelling approach followed a progressive framework beginning with a crude model evaluating plasmid type alone, followed by an additive model including continent as a covariate, and finally an interaction model to explore effect modification by continent.

Due to the dataset's distribution, several plasmid types exhibited limited variability in beta-lactamase carriage. As shown in the earlier distribution (Figure 3), some plasmid types carry no antimicrobial resistance genes, while a subset (e.g. 333) consistently carry beta-lactamase genes in 100% of observed cases. These extreme imbalances led to complete or near-complete separation in the logistic regression models, which in turn resulted in inflated odds ratios and wide or unstable confidence intervals for affected plasmid categories, even after applying robust standard errors. Firth's penalized logistic regression was explored as a sensitivity check, as it is known to address issues of separation by applying bias reduction. While it provided more conservative estimates in specific cases like 333, it could not accommodate interaction terms in the current dataset and was therefore not used as the main modeling approach. Given these modelling constraints, interpretation of results focuses primarily on the direction and relative strength of associations rather on precise effect estimates, particularly for plasmid types with limited variation in resistance profile. (All raw model outputs are provided in the Supplementary Results.)

Across all outcomes (beta-lactamase, ESBL, and carbapenemase gene carriage), model fit progressively improved with each model, and the interaction model consistently provided the best fit (AIC = 6086.8 for beta-lactamase, 5929.0 for ESBL, and 5621.6 for carbapenemase). A subset of plasmid types, particularly types 68, 75, 94, 276, 333, and 486, were repeatedly identified as strong predictors of resistance gene carriage. In the crude and additive models, several of these types were significantly associated with higher odds of gene presence, with slight variations in direction and magnitude depending on the gene category. The additive models further showed that isolates from Europe, North America, and Oceania often had lower odds of resistance gene carriage compared to those from Asia, although this varied by outcome.

Model comparison using likelihood ratio tests confirmed that the interaction models significantly improved model fit over the additive models for the three outcomes. The inclusion of the interaction term between plasmid type and continent significantly reduced model deviance for beta-lactamase carriage (Deviance = 198.5, df = 96, $p < 0.001$) and similarly for ESBL (Deviance = 266.9, df = 96, $p < 0.001$) and carbapenemase carriage (Deviance = 387.8, df = 96, $p < 0.001$), supporting the selection of the interaction model as the final model for interpretation in each case.

The interaction models revealed substantial region-specific variation in plasmid–resistance gene associations. For beta-lactamase carriage, strong positive associations were observed in Asia across most key plasmid types, with significantly stronger effects for types 276, 68, and 94 in Africa, and for types 276 and 75 in Europe. Oceania also showed stronger associations for types 276, 68, 75, and 94 compared to Asia. Conversely, North America and South America often showed weaker and negative interaction estimates for these plasmids, with statistically significant negative interaction terms for types 68 and 94 in North America.

For ESBL carriage, although the crude model suggested several negative associations, plasmid types 68, 75, and 94 consistently remained to have a positive association. In Asia, positive associations with ESBL carriage were observed for plasmid types 68 and 486, while type 333 remained strongly negatively associated. Europe exhibited stronger positive associations for types 68 and 94 but a significantly negative association for type 75. In Oceania, all three plasmid types shown to have a positive association in the crude model (68, 75, and 94) were linked to increased odds of ESBL carriage. Meanwhile in North America showed a significantly negative association for type 276. South America again showed mostly weak or attenuated effects, while in Africa, no statistically significant associations were observed between any plasmid types and ESBL carriage.

In the case of carbapenemase carriage, the pattern of associations echoed those observed for beta-lactamase. In Asia, plasmid types 276, 333, 486, 68, and 94 were significantly associated with increased odds of carriage. Region-specific variations were again notable. In Europe, plasmid types 276, 75, and 68 showed elevated associations, while type 486 displayed a significantly weaker effect. In Oceania, plasmid types 276, 68, and 75 were also linked to stronger positive associations, while type 94 showed a slightly reduced effect. In contrast, several key plasmid types were significantly associated with lower odds of carbapenemase carriage in North and South America. Specifically, in South America, plasmid types 276, 75, and 94 exhibited statistically significant negative associations. Africa, once again, showed no statistically significant associations between plasmid type and carbapenemase carriage.

Given the observed regional differences in plasmid-type associations with carbapenemase carriage particularly the strong signals detected in Asia and Europe, a stratified analysis was conducted for these two regions to further explore the patterns identified in the residual analysis. In Asia, plasmid types 333 and 94 were most strongly associated with carbapenemase carriage compared to type 486 and other plasmid types. Type 333 showed an especially extreme effect, while type 94 displayed a moderately strong but statistically significant association. In Europe, where plasmid type 276 served as the reference due to its previously established relevance, no other plasmid type showed a stronger association. Instead, all remaining types were associated with significantly lower odds of carbapenemase carriage, suggesting a distinct regional distribution and potential dominance of type 276 in this setting. Although plasmid type 333 yielded a positive and statistically significant estimate relative to 276, its limited presence in European isolates likely reflects sparse data and should be interpreted with caution.

Structural Comparison of Key Carbapenemase-Associated Plasmids

Following the strong regional associations observed for plasmid types 333 and 276 in both the residual analysis and regression models, sequence alignment was performed to investigate whether the plasmids associated with carbapenemase carriage in different regions are genetically distinct. The alignment showed that these two plasmids do not share any conserved backbone regions apart from the blaOXA-48-like carbapenemase gene. The plasmid associated with Asia (type 333) appeared to be smaller and mobilizable (pMOB), while the plasmid associated with Europe (type 276) was larger and encoded a complete set of conjugation machinery, including multiple tra genes, suggesting it is conjugative (pCONJ). These differences indicate that plasmid 276 in Europe may be more autonomous in its ability to transfer between bacterial hosts, whereas plasmid 333 in Asia likely relies on co-resident

conjugative elements for mobilization. This structural difference may contribute to the distinct transmission dynamics observed across regions.

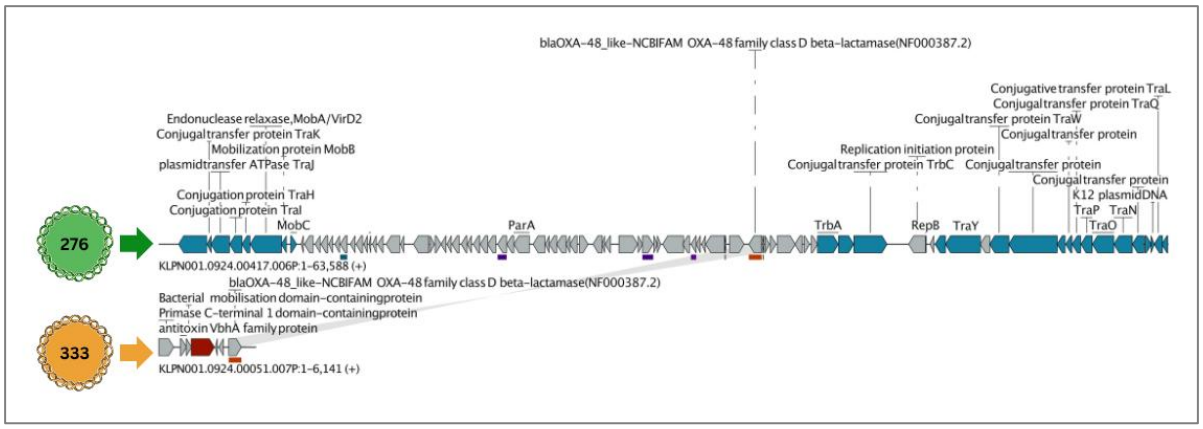

Figure 11: Sequence Alignment of Key Carbapenemase – Associated Plasmids

DISCUSSION

This study examined how plasmids contribute to the spread of beta-lactamase genes by analyzing a global collection of 2,074 high-quality *Klebsiella pneumoniae* genome assemblies from the NCBI RefSeq database. The primary aims were to describe the distribution of resistance genes, identify plasmid types commonly associated with beta-lactamase carriage, and explore whether certain plasmid types show geographic patterns that may influence their regional spread. Particular attention was given to two clinically important beta-lactamase subgroups, extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which are recognized by the World Health Organization as critical targets for antimicrobial resistance surveillance and control (15).

Distribution results were mostly in line with what previous studies have shown. A large majority (68.7%) of all antibiotic resistance genes (ARGs) in the dataset were found on plasmids (20,42). Among these, beta-lactamase genes stood out as both the most frequent and most diverse class, with 126 different variants. Still, despite this diversity, more than 90 percent of these genes belonged to just six main families: *blaTEM*, *blaCTX-M*, *blaOXA*, *blaKPC*, *blaSHV*, and *blaNDM*. These families are known to include genes that produce extended-spectrum beta-lactamases (ESBLs) and carbapenemases (Figure 2) (13,14). Among them, ESBL-associated families appeared more frequently, suggesting that ESBLs may be playing a broader role in the current plasmid-mediated resistance landscape. This supports the idea that *K. pneumoniae* remains a major reservoir for beta-lactamase genes and that plasmids continue to play a central role in their spread.

In total, 7,147 plasmids were identified across the dataset. While more than half carried at least one resistance gene, only 39.65% were found to encode beta-lactamase genes specifically.

These beta-lactamase-carrying plasmids were generally larger in size and conjugative, they therefore possess the full set of genes required for autonomous transfer between bacterial cells. This structural feature likely plays a role in facilitating the spread of resistance. Among all plasmids, 224 distinct plasmid types were identified, but only 94 were linked to beta-lactamase carriage. Of these, the majority (62 types) were shared across different resistance subgroups, carrying either ESBL genes, carbapenemase genes, or both. This pattern aligns with earlier findings showing that a relatively small number of plasmid lineages are responsible for spreading most of the clinically significant resistance genes (43,44).

Given that only a small number of plasmid lineages carry the majority of clinically relevant resistance genes suggests a potentially useful focus for surveillance. If these lineages consistently appear across different contexts, they could be used to track the spread of resistance more effectively. However, commonly used classification systems, such as incompatibility (Inc) groups and plasmid taxonomic units (PTUs), offer limited resolution. While useful for broad categorization, they often fail to capture the finer structure of plasmid diversity and do not always reflect resistance gene carriage patterns in detail. To address this limitation, this study used an alternative way to classify plasmids using an in-house typing framework which grouped plasmids based on genetic similarity thresholds and specific sequence markers. This allowed for a more precise grouping of plasmids and offered a clearer working definition of plasmid types that may be more relevant for understanding resistance gene dissemination.

To explore how these plasmid lineages contribute to resistance spread, statistical tests were conducted to assess whether specific plasmid types were associated with the presence of beta-lactamase as well as ESBL and carbapenemase genes. The results showed significant associations, indicating that some plasmid types were more likely to carry specific resistance genes than others, even though earlier findings had shown that most plasmid types are shared across resistance groups. For instance, types 75 and 486 appeared more frequently among plasmids carrying ESBL genes, while types 276 and 333 were more often linked to carbapenemase carriage. One type in particular, plasmid 333, stood out as it was never observed in ESBL-associated plasmids and was found exclusively with carbapenemases. This exclusive link suggests that plasmid type 333 could serve as a useful marker for high-risk resistance and may have value in targeted surveillance efforts.

The analysis then examined whether plasmid types were also associated with specific geographic regions. Statistically significant associations were found between plasmid type and continent of isolation, both for beta-lactamase genes in general, and for the ESBL and carbapenemase subsets. These findings suggest that certain plasmid types not only tend to

carry specific resistance genes but may also be more prevalent in particular regions, depending on the resistance subtype. Residual analyses helped highlight which plasmid types were more common than expected in specific regions, depending on whether they carried beta-lactamase or a specific subtype. Although most plasmid types were observed across multiple continents, certain lineages showed clear geographic enrichment, especially when resistance subgroups were examined separately.

Plasmid type 486, the most frequent in the dataset and commonly linked to beta-lactamase carriage, was significantly enriched in Asia, particularly in plasmids carrying ESBL genes. Although it appeared in other regions and resistance groups, its strong ESBL association in Asia suggests that it may play an important role in driving resistance within that region. In contrast, plasmid type 333, though less common overall, was found exclusively in carbapenemase-carrying plasmids and was observed almost entirely within Asia. This strong, and exclusive association highlights type 333 as a potentially high-risk lineage for carbapenemase spread in the region. The presence of types 486 and 333 in Asia also supports earlier findings that the region is a key reservoir for beta-lactamase–associated plasmids, and a hotspot for multidrug-resistant *strains*. Environmental studies have also pointed to a broader reservoir of resistance genes, reinforcing the need for sustained genomic surveillance, particularly in low- and middle-income settings where AMR is expected to rise (45,46).

In Europe, plasmid type 276 was the most enriched for overall beta-lactamase gene carriage. However, when resistance subgroups were examined separately, it was more strongly associated with carbapenemase genes in Europe and with ESBL genes in Africa. While a mild association with Africa was already apparent in the beta-lactamase analysis, the ESBL-specific residuals showed a much stronger signal and were nearly absent in Europe. This shift suggests that the same plasmid type may carry different resistance genes depending on regional or ecological contexts.

Plasmid type 68 also showed region-specific patterns. While it was moderately enriched in Europe and Africa overall for beta-lactamase carriage, its strongest association for ESBL carriage was in Oceania. Meanwhile, within the carbapenemase subset, type 68 was more strongly linked to Africa. These findings suggest that in Oceania, type 68 likely carries ESBL genes, whereas in Africa it may be more involved in the spread of carbapenemase resistance. Given this observation, Oceania may currently be experiencing a predominantly ESBL driven resistance burden. Recent evidence from the region, particularly Australia have documented the presence of ESBL-associated genes such as *blaCTX-M* and *blaTEM* in K. pneumoniae, alongside rising rates of ESBL-producing E. coli in Central Australia. These trends have been linked to factors including high antibiotic usage, socioeconomic disadvantage, and frequent

healthcare exposure (47,48). The exclusive association of plasmid type 68 with ESBLs in Oceania, and its absence from the carbapenemase-specific analysis, may indicate that carbapenem resistance has not yet widely disseminated in the region through plasmids, offering a possible window for early intervention or containment.

Type 94 showed a different pattern altogether. Although its residual signals per continent were weaker than those of types 486, 333, or 276, it stood out for its apparent plasticity. Unlike other types, it was not clearly associated with either ESBL or carbapenemase genes alone. Instead, it appeared across both groups, suggesting it may adapt more flexibly depending on the local resistance landscape. In terms of regional trends, type 94 was linked with ESBL carriage in Europe and North America, but not in Asia. For carbapenemase genes, it showed a stronger presence in South America. This variability makes it a plasmid type worth monitoring, especially for its potential to bridge resistance groups across different settings.

Logistic regression models reinforced the findings from the residual analyses, particularly through final stratified models for Asia and Europe, where earlier results had shown strong geographic signals. The patterns became more distinct, showing that plasmid type 333 was significantly associated with carbapenemase carriage in Asia, while type 276 showed a similar association in Europe. To explore whether these types represented genuinely distinct plasmid lineages, representative sequences were aligned. The comparison revealed clear structural differences between the two plasmids, supporting the framework's suitability for grouping plasmids and its ability to capture meaningful variation. Type 276, which was prevalent in Europe, was found to be large and conjugative, capable of transferring independently between bacteria, whereas type 333 in Asia was smaller and mobilizable, likely relying on co-resident plasmids to facilitate transfer. These structural differences may influence how easily each type spreads, but both represent high-risk plasmid lineages with important public health implications.

LIMITATIONS

While this study offers important insights into plasmid-mediated beta-lactamase dissemination in *Klebsiella pneumoniae*, several limitations should be taken into account when interpreting the findings. The genomic dataset was drawn from publicly available RefSeq assemblies, which may be subject to regional sampling imbalances. Countries with greater sequencing capacity or more established surveillance programs are often overrepresented, while low-resource settings remain under-sampled and often lack sufficient data. As a result, the observed geographic patterns may reflect differences in sequencing volume rather than true biological distribution, limiting the generalizability of the results to underrepresented regions.

To ensure geographic comparability, analyses were conducted at the continent level due to inconsistencies in the reporting of more specific sampling locations. While this allowed for broader comparisons, a finer-grained geographic context would provide more actionable insights and may help reveal transmission dynamics that remain undetectable at the continental scale.

The statistical models used also showed signs of instability influenced by data separation or limited variability in certain plasmid types. While appropriate steps were taken to address this, results should be interpreted with caution, focusing on overall patterns rather than precise effect sizes.

Although metadata on collection year was available, temporal trends were not analyzed in this study. This limits the ability to determine whether particular plasmid types are newly emerging, declining, or persisting over time which is an important aspect of understanding resistance evolution and potential public health risk.

Another key limitation is the absence of phylogenetic correction for bacterial lineage. Without incorporating a core genome phylogeny, it is difficult to assess whether observed plasmid–resistance associations are the result of horizontal gene transfer or clonal expansion. This also limits the ability to infer transmission routes or evolutionary origins, including the processes by which plasmids may acquire or lose resistance genes across lineages.

Finally, this study did not include data on regional antibiotic usage. Antibiotic pressure is a major driver of AMR evolution and plasmid selection, and its absence represents a significant limitation. Without it, the influence of local antimicrobial consumption patterns on the distribution of resistance plasmids cannot be directly assessed, leaving an important gap in understanding the ecological factors that shape resistance dynamics.

FUTURE DIRECTIONS

Building on the current findings, several future directions could deepen the understanding of plasmid-mediated beta-lactamase dissemination in *K. pneumoniae*. A key next step is to incorporate a core genome phylogeny to account for bacterial population structure. Comparing plasmid distribution patterns against the phylogenetic relationships of host genomes would help disentangle the contributions of horizontal gene transfer versus clonal expansion. This approach would allow for more accurate inference about the evolutionary dynamics of plasmid types and clarify whether observed geographic trends reflect genuine plasmid mobility or the spread of resistant bacterial lineages.

Adding a temporal dimension to the analysis is also an important priority. Although collection dates were available, they were not used in this study. Future research could track the emergence, persistence, or decline of key plasmid types over time, providing valuable insights into resistance trajectories. This may help identify early warning signals, detect shifts in resistance patterns, and distinguish between newly emerging threats and long-standing reservoirs of resistance.

Finally, integrating data on regional antibiotic usage could help link plasmid-associated resistance patterns to the selective pressures that drive them. Correlating gene carriage with antimicrobial consumption across regions may reveal ecological drivers of plasmid success and support the design of more tailored antibiotic stewardship strategies. Taken together, combining molecular epidemiology with public health surveillance can offer a more comprehensive framework for anticipating and mitigating the global spread of antimicrobial resistance.

CONCLUSION

Altogether, these findings highlight the context-dependent nature of plasmid-mediated resistance. The same plasmid type may be linked to different resistance genes depending on geographic and ecological factors, as seen with types like 94 and 276. Such variation suggests that local antibiotic use, healthcare practices, and transmission dynamics could influence which resistance traits are maintained and spread

The wider geographic spread of ESBL-associated plasmids, compared to the more localized distribution of carbapenemase-linked ones, may reflect differences in how long these plasmids have been circulating or how readily they spread, though these dynamics remain poorly understood and were not directly explored in this study. In some regions, such as Oceania, ESBL-associated plasmids like type 68 appear to be dominant without evidence of carbapenemase gene carriage. This could represent a crucial period for intervention before further resistance traits are acquired. Similarly, the restricted geographic presence of certain carbapenemase plasmids, such as type 333 in Asia, might indicate early stages of spread, offering another opportunity for targeted containment. Continued genomic surveillance will be essential to monitor these trends and support timely public health responses.

Lasty, Integrating detailed plasmid typing with regional data can offer a practical framework for strengthening genomic surveillance. Knowing which plasmid types circulate in specific areas, and what resistance genes they tend to carry, could help anticipate and respond to emerging threats. While many factors still remain to be clarified, this approach may help identify early warning signals and refine surveillance efforts in regions where AMR is rapidly evolving.

SUPPLEMENTARY RESULTS AND MATERIALS

A. Sequence Type Distribution

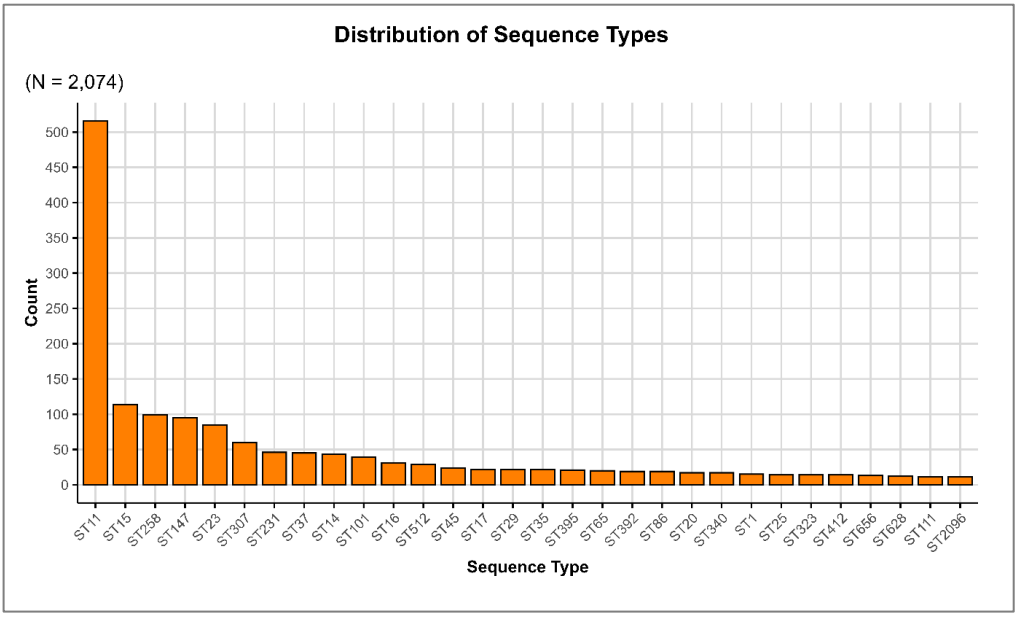

Figure S1: Distribution of Sequence Types
The figure shows the distribution of the top 30 ST in the dataset by decreasing frequency.

A total of 351 unique sequence types (STs) were identified using Kleborate, though most were rare, each contributing less than 1% of isolates. A small subset accounted for the majority, with ST11 being the most dominant (24.8%), and the top five STs collectively representing 43.8% of the dataset.

Key *K. pneumoniae* STs previously identified through MLST include ST11, ST14, ST15, ST26, ST101, ST147, ST149, ST231, ST258, ST627, and ST977, each showing distinct geographic and epidemiological trends. ST258 is mainly found in North America, Latin America, and Europe, while ST11 is more common in Asia and South America. ST627 has been reported in both clinical and poultry sources across Egypt, Korea, Greece, and Spain, raising concerns about zoonotic transmission (49).

In Vietnam, ST23 is the predominant hypervirulent type (hvKp), frequently linked to liver abscesses and reported globally. Carbapenem-resistant ST23 strains have also emerged in China. ST86 and ST65 are the next most common hvKp types in Vietnam, with ST65 also noted in China. ST25, a major carrier of virulence plasmids like *iuc3*, has been reported in Vietnam and the US, with carbapenem-resistant ST25 strains now observed in China (22).

B. Summary of Drug Classes and Associated Resistance Genes

➢ B.1. Genome – Level Summary

At the genome level, beta-lactam resistance genes showed the greatest diversity, followed by aminoglycoside genes. Together, beta-lactam, aminoglycoside, and phenicol/quinolone classes accounted for over half of all identified resistance genes.

Supplementary Table S1. Antibiotic Resistance Genes by Class (genome-level summary)

Summary of unique genes grouped by the type of resistance conferred. (N = 29,778)

Drug Class	Genes	Total Unique Genes	Total Observations	
			n	Percent %
BETA-LACTAM	blaTEM-1, blaSHV-11, blaCTX-M-15, blaKPC-2, blaOXA-1, blaSHV-1, blaSHV-12, blaCTX-M-65, blaSHV-28, blaNDM-1, blaDHA-1, blaLAP-2, blaCTX-M-14, blaOXA-48, blaKPC-3, blaOXA-9, blaNDM-5, blaOXA-232, blaCTX-M-3, blaSHV, blaOXA-10, blaOXA-181, blaSHV-27, blaCTX-M, blaCTX-M-55, blaCTX-M-27, blaTEM, blaOXA, blaTEM-31, blaSHV-26, blaKPC-33, blaSHV-33, blaCMY-2, blaCTX-M-2, blaSHV-5, blaVIM-1, blaCMY-4, blaIMP-4, blaSHV-2A, blaCMY-16, blaCMY-6, blaSHV-75, blaSFO-1, blaNDM-7, blaSCO-1, blaSHV-36, blaSHV-110, blaSHV-168, blaSHV-187, blaKPC-14, blaKPC-31, blaNDM-4, blaSHV-142, blaVEB-1, blaCTX-M-24, blaKPC-12, blaKPC-90, blaSHV-108, blaSHV-2, blaSHV-7, blaSHV-71, bla, blaCMY, blaCTX-M-90, blaOXA-2, blaSHV-207, blaSHV-31, blaKPC, blaKPC-35, blaSHV-205, blaSHV-25, blaSHV-30, blaSHV-32, blaSHV-41, blaTEM-2, blaCTX-M-9, blaFONA, blaIMP-1, blaKPC-17, blaKPC-189, blaKPC-71, blaKPC-8, blaKPC-93, blaNDM-14, blaNDM-3, blaSHV-119, blaSHV-217, blaSHV-232, blaSHV-38, blaSHV-61, blaSHV-76, blaTEM-135, blaTEM-169, blaTEM-190, blaVEB-3, blaVIM-27, blaACC-1, blaCARB-2, blaCMY-172, blaCMY-174, blaCMY-23, blaCTX-M-1, blaCTX-M-104, blaCTX-M-125, blaCTX-M-25, blaCTX-M-62, blaCTX-M-63, blaCTX-M-71, blaDHA, blaDHA-15, blaFOX-5, blaIMP-26, blaIMP-38, blaIMP-68, blaIMP-8, blaKPC-103, blaKPC-105, blaKPC-106, blaKPC-107, blaKPC-108, blaKPC-109, blaKPC-110, blaKPC-125, blaKPC-129, blaKPC-139, blaKPC-140, blaKPC-142, blaKPC-143, blaKPC-144, blaKPC-228, blaKPC-44, blaKPC-53, blaKPC-66, blaKPC-68, blaKPC-74, blaKPC-78, blaKPC-81, blaKPC-86, blaLAP, blaMOX, blaNDM-19, blaNDM-6, blaOXA-204, blaOXA-21, blaOXA-244, blaPER-1, blaPER-7, blaSHV-144, blaSHV-157, blaSHV-186, blaSHV-214, blaSHV-226, blaSHV-229, blaSHV-37, blaSHV-40, blaSHV-42, blaSHV-44, blaSHV-45, blaSHV-60, blaSHV-77, blaTEM-206, blaTEM-210, blaTEM-26, blaVEB-8, blaVIM-4	165	8168	27.43
AMINOGLYCOSIDE	aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(6')-Ib, aac(3)-IId, rmtB1, aac(3)-IIe, armA, aadA16, aadA1, aac(3)-IVa, aph(4)-Ia, aph(3')-VI, rmtF1, aac(6')-Ib3, ant(2'')-Ia, aadA5, aadA2, aac(6')-Ib4, aac(6')-Ib', rmtC, aph(3')-IIa, aph(3')-VIb, aac(3)-IIg, aac(6')-IIc, aac(6')-II, aph(3')-VIa, aac(6')-33, aph(3')-XV, aac(3)-Ia, aac(6')-Im, aph(2'')-IIa, rmtG, aac(3)-VIa, aac(6')-IIa, aacA16, aph(3')-II, rmtF, rmtH, aac(6'), aac(6')-Ia, aac(6')-Ian, aac(6')-Ib11, aac(6')-Iq, aadA8, aph(3')-IIb, aph(6)-Ic, aph(7'')-Ia, gar, rmtF2	50	5028	16.88

PHENICOL/ QUINOLONE	oqxA, oqxB, oqxB19, oqxB25, oqxA10, oqxA11, oqxB32, oqxB5, oqxB20, oqxA3, oqxB6, oqxB14, oqxB17, oqxA2, oqxB21, oqxA6, oqxA4, oqxB10, oqxB27, oqxB2	20	3459	11.62
SULFONAMIDE	sul1, sul2, sul3	3	2575	8.65
FOSFOMYCIN	fosA, fosA3, fosA10, fosA5, fosA8, fosA7, fosE	7	2193	7.36
TRIMETHOPRIM	dfrA14, dfrA12, dfrA50, dfrA27, dfrA1, dfrA5, dfrA17, dfrA51, dfrA30, dfrA19, dfrA15, dfrA25, dfrA23, dfrA7, dfrA35, dfrA32, dfrA8, dfrA16, dfrA4, dfrB1, dfrA6	21	1769	5.94
TETRACYCLINE	tet(A), tet(D), toprJ1, tmexD1, tmexC1, tet(G), tet(B), tet(X4), tmexC2, toprJ2, tet(M), tmexD2, tmexD, tmexC, tet(C), tmexC3	16	1286	4.32
PHENICOL	floR, catA2, catA1, catB3, cmlA1, catB, cmlA5, floR2, cmlA10, catB2, cmlA4, catB11, catB8, cmlA, cmx	15	1231	4.13
QUINOLONE	qnrS1, qnrB1, qnrB4, qnrB91, qnrB52, qnrB6, qnrB, qnrB19, qnrA1, qnrB2, qnrB9, qnrA6, qepA1, qnrA3, qepA, qnrE2, qnrS, qnrVC4	18	1177	3.95
MACROLIDE	mph(A), mph(E), ere(A), estT, mef(B), erm, ere(B)	7	900	3.02
AMINOGLYCOSIDE/ QUINOLONE	aac(6')-Ib-cr5, aac(6')-Ib-cr	2	706	2.37
RIFAMYCIN	arr-3, arr-2, arr	3	517	1.74
BLEOMYCIN	ble, bleO	2	324	1.09
MACROLIDE/ STREPTOGRAMIN	msr(E)	1	204	0.69
COLISTIN	mcr-1.1, mcr-8.1, mcr-8.2, mcr-10.1, mcr-3.21, mcr-1.32, mcr-1.26, mcr-2.3, mcr-3, mcr-3.22, mcr-3.26, mcr-3.28, mcr-3.40, mcr-8.3	14	73	0.25
LINCOSAMIDE/ MACROLIDE/ STREPTOGRAMIN	erm(B), erm(T)	2	64	0.21
STREPTOTHRICIN	sat2	1	62	0.21
LINCOSAMIDE/ MACROLIDE	erm(42)	1	28	0.09
LINCOSAMIDE	lnu(F), lnu(G)	2	13	0.04
PHENICOL/ LINCOSAMIDE/ OXAZOLIDINONE/ PLEUROMUTILIN/ STREPTOGRAMIN	cfr	1	1	0.00

➢ B.2. Plasmid – Specific Summary

Supplementary Table S2. Antibiotic Resistance Genes by Class (plasmid-specific summary)

Summary of unique genes grouped by the type of resistance conferred (N = 20,459)

Drug Class	Genes	Total Unique Genes	n	Percent %
BETA-LACTAM	blaTEM-1, blaKPC-2, blaCTX-M-15, blaOXA-1, blaSHV-12, blaCTX-M-65, blaNDM-1, blaLAP-2, blaDHA-1, blaOXA-48, blaCTX-M-14, blaKPC-3, blaOXA-9, blaNDM-5, blaOXA-232, blaCTX-M-3, blaSHV-11, blaOXA-10, blaOXA-181, blaCTX-M-55, blaCTX-M, blaTEM, blaTEM-31, blaOXA, blaCTX-M-27, blaKPC-33, blaCMY-2, blaSHV-1, blaCTX-M-2, blaVIM-1, blaIMP-4, blaSHV-2A, blaCMY-16, blaCMY-4, blaCMY-6, blaSFO-1, blaSHV-5, blaNDM-7, blaSCO-1, blaKPC-14, blaKPC-31, blaNDM-4, blaVEB-1, blaCTX-M-24, blaKPC-12, blaKPC-90, blaSHV-2, blaSHV-7, bla, blaCMY, blaCTX-M-90, blaOXA-2, blaKPC, blaKPC-35, blaSHV-30, blaSHV-31, blaTEM-2, blaCTX-M-9, blaFONA, blaIMP-1, blaKPC-17, blaKPC-189, blaKPC-71, blaKPC-8, blaKPC-93, blaNDM-14, blaTEM-135, blaTEM-190, blaVEB-3, blaVIM-27, blaACC-1, blaCARB-2, blaCMY-172, blaCMY-174, blaCMY-23, blaCTX-M-1, blaCTX-M-104, blaCTX-M-125, blaCTX-M-25, blaCTX-M-63, blaCTX-M-71, blaDHA, blaDHA-15, blaFOX-5, blaIMP-26, blaIMP-38, blaIMP-68, blaIMP-8, blaKPC-103, blaKPC-105, blaKPC-106, blaKPC-107, blaKPC-108, blaKPC-109, blaKPC-110, blaKPC-125, blaKPC-129, blaKPC-139, blaKPC-140, blaKPC-142, blaKPC-143, blaKPC-228, blaKPC-44, blaKPC-53, blaKPC-66, blaKPC-68, blaKPC-74, blaKPC-78, blaKPC-81, blaKPC-86, blaLAP, blaMOX, blaNDM-19, blaNDM-6, blaOXA-204, blaOXA-21, blaOXA-244, blaPER-1, blaSHV, blaSHV-28, blaSHV-44, blaTEM-169, blaTEM-206, blaTEM-210, blaTEM-26, blaVEB-8	126	5496	26.86
AMINOGLYCOSIDE	aph(6)-Id, aph(3'')-Ib, aph(3')-Ia, aac(6')-Ib, rmtB1, aac(3)-IId, aac(3)-IIe, aadA16, armA, aadA1, aac(3)-IVa, aph(3')-VI, aph(4)-Ia, rmtF1, aac(6')-Ib3, ant(2'')-Ia, aadA5, aac(6')-Ib4, aac(6')-Ib', aadA2, aph(3')-IIa, rmtC, aph(3')-VIb, aac(6')-Il, aac(3)-IIg, aac(6')-IIc, aph(3')-VIa, aac(6')-33, aac(3)-Ia, aac(6')-Im, aph(2'')-IIa, aph(3')-XV, rmtG, aac(3)-VIa, aacA16, aph(3')-II, rmtF, rmtH, aac(6'), aac(6')-Ia, aac(6')-Ian, aac(6')-Ib11, aac(6')-Iq, aph(6)-Ic, aph(7'')-Ia, gar, rmtF2	47	4677	22.86
SULFONAMIDE	sul1, sul2, sul3	3	2365	11.56
TRIMETHOPRIM	dfrA14, dfrA12, dfrA50, dfrA27, dfrA1, dfrA5, dfrA17, dfrA51, dfrA30, dfrA19, dfrA15, dfrA25, dfrA23, dfrA35, dfrA8, dfrA32, dfrA4, dfrB1, dfrA16, dfrA6, dfrA7	21	1639	8.01
TETRACYCLINE	tet(A), tet(D), toprJ1, tmexD1, tmexC1, tet(G), tet(B), tet(X4), tmexC2, toprJ2, tet(M), tmexD2, tmexD, tmexC, tet(C), tmexC3	16	1224	5.98
PHENICOL	catA2, floR, catA1, catB3, cmlA1, catB, cmlA5, floR2, catB2, cmlA4, catB11, catB8	12	1172	5.73
QUINOLONE	qnrS1, qnrB1, qnrB4, qnrB91, qnrB6, qnrB, qnrB52, qnrB19, qnrA1, qnrB2, qnrB9, qnrA6, qepA1, qnrA3, qepA, qnrE2, qnrS, qnrVC4	18	1131	5.53
MACROLIDE	mph(A), mph(E), ere(A), estT, mef(B), erm, ere(B)	7	848	4.14
AMINOGLYCOSIDE/ QUINOLONE	aac(6')-Ib-cr5, aac(6')-Ib-cr	2	609	2.98
RIFAMYCIN	arr-3, arr-2, arr	3	492	2.40
BLEOMYCIN	ble, bleO	2	306	1.50
MACROLIDE/ STREPTOGRAMIN	msr(E)	1	183	0.89
FOSFOMYCIN	fosA3, fosA, fosA8, fosE	4	119	0.58

COLISTIN	mcr-8.1, mcr-1.1, mcr-8.2, mcr-10.1, mcr-3.21, mcr-1.26, mcr-2.3, mcr-3, mcr-3.22, mcr-3.26, mcr-3.28, mcr-3.40, mcr-8.3	13	70	0.34
LINCOSAMIDE/ MACROLIDE/ STREPTOGRAMIN	erm(B), erm(T)	2	58	0.28
LINCOSAMIDE/ MACROLIDE	erm(42)	1	28	0.14
STREPTOTHRICIN	sat2	1	18	0.09
LINCOSAMIDE	lnu(F), lnu(G)	2	12	0.06
PHENICOL/ QUINOLONE	oqxA2, oqxB, oqxA, oqxB2	4	11	0.05
PHENICOL/ LINCOSAMIDE/ OXAZOLIDINONE/ PLEUROMUTILIN/ STREPTOGRAMIN	cfr	1	1	0.00

C. Logistic Regression Results

➢ C.1. Beta-Lactamase Models

Beta-Lactamase Presence					
Crude Model [Beta ~ Plasmid Type]					
Null deviance: **9593.7** on 7146 degrees of freedom Residual deviance: **6124.3** on 7127 degrees of freedom AIC: **6164.3**					
Plasmid Type	**Estimate**	**OR**	**CI (low)**	**CI (high)**	**P-value**
(Intercept)	-0.72	0.49	0.45	0.53	**<0.001**
1063	-17.85	0.00	0.00	0.00	**<0.001**
1105	1.22	3.38	2.61	4.37	**<0.001**
209	2.87	17.70	10.69	29.30	**<0.001**
2212	0.72	2.05	1.42	2.95	**<0.001**
251	-2.58	0.08	0.04	0.15	**<0.001**
276	3.21	**24.70**	14.52	42.04	**<0.001**
291	-17.85	0.00	0.00	0.00	**<0.001**
333*	19.28	**236758778.73**	183759401.66	305044089.17	**<0.001**
410	-17.85	0.00	0.00	0.00	**<0.001**
420	-3.01	0.05	0.02	0.10	**<0.001**
449	0.66	1.93	1.50	2.48	**<0.001**
463	2.28	9.79	6.21	15.43	**<0.001**
486	4.05	**57.32**	32.79	100.20	**<0.001**
52	-1.23	0.29	0.18	0.48	**<0.001**
53	-17.85	0.00	0.00	0.00	**<0.001**
542	-4.56	0.01	0.00	0.07	**<0.001**
68	2.17	**8.77**	6.12	12.55	**<0.001**
75	3.65	**38.44**	19.60	75.39	**<0.001**
94	3.48	**32.53**	16.54	63.97	**<0.001**

Beta-Lactamase Presence

Plasmid Type	Additive Model [Beta ~ Plasmid Type + Continent]					Interaction Model [Beta ~ Plasmid Type * Continet]				
	Null deviance: **9593.7** on 7146 degrees of freedom Residual deviance: **6041.2** on 7121 degrees of freedom AIC: **6093.2**					Null deviance: **9593.7** on 7146 degrees of freedom Residual deviance: **5842.8** on 7025 degrees of freedom AIC: **6086.8**				
	Estimate	**OR**	**CI (low)**	**CI (high)**	**P-value**	**Estimate**	**OR**	**CI (low)**	**CI (high)**	**P-value**
Intercept	-0.56	0.57	0.51	0.63	**<0.001**	-0.57	0.57	0.51	0.63	**<0.001**
1063	-17.80	0.00	0.00	0.00	**<0.001**	-18.00	0.00	0.00	0.00	**<0.001**
1105	1.18	3.26	2.53	4.21	**<0.001**	1.53	4.64	3.17	6.77	**<0.001**
209	2.73	15.37	9.26	25.49	**<0.001**	2.97	19.51	10.75	35.39	**<0.001**
2212	0.77	2.15	1.49	3.12	**<0.001**	0.93	2.53	1.46	4.37	**<0.001**
251	-2.62	0.07	0.04	0.14	**<0.001**	-3.22	0.04	0.01	0.13	**<0.001**
276	3.24	**25.43**	14.70	43.97	**<0.001**	2.53	**12.57**	5.66	27.91	**<0.001**
291	-17.85	0.00	0.00	0.00	**<0.001**	-18.00	0.00	0.00	0.00	**<0.001**
333*	19.19	**216443126.48**	166883767.03	280720095.40	**<0.001**	19.13	**203533525.96**	148652809.43	2.78675500.00	**<0.001**
410	-18.00	0.00	0.00	0.00	**<0.001**	-18.00	0.00	0.00	0.00	**<0.001**
420	-3.14	0.04	0.02	0.09	**<0.001**	-3.16	0.04	0.02	0.00	**<0.001**
449	0.64	1.89	1.47	2.43	**<0.001**	0.35	1.42	0.99	2.04	**0.055**
463	2.24	9.37	5.88	14.95	**<0.001**	1.66	5.28	3.07	9.07	**<0.001**
486	3.90	**49.39**	28.16	86.64	**<0.001**	4.14	**63.01**	33.37	118.95	**<0.001**
52	-1.25	0.29	0.17	0.48	**<0.001**	-0.79	0.45	0.22	0.95	**0.036**
53	-18.00	0.00	0.00	0.00	**<0.001**	-18.00	0.00	0.00	0.00	**<0.001**
542	-4.57	0.01	0.00	0.07	**<0.001**	-18.00	0.00	0.00	0.00	**<0.001**
68	2.31	**10.07**	6.77	14.98	**<0.001**	2.15	**8.55**	4.76	15.34	**<0.001**
75	3.66	**39.04**	19.62	77.67	**<0.001**	3.74	**42.24**	15.49	115.16	**<0.001**
94	3.47	**32.28**	16.34	63.77	**<0.001**	4.61	**100.32**	13.91	723.66	**<0.001**
Africa	0.23	1.26	0.80	2.00	0.317	-0.23	0.79	0.34	1.82	0.583
Europe	-0.22	0.80	0.69	0.94	**<0.01**	-0.26	0.77	0.65	0.93	**0.005**
North America	-0.29	**0.75**	0.60	0.93	**<0.01**	-0.20	**0.82**	0.65	1.05	0.113
Oceania	-1.40	**0.25**	0.18	0.33	**<0.001**	-1.75	**0.17**	0.10	0.30	**<0.001**
South America	-0.02	0.98	0.69	1.41	0.923	0.15	1.16	0.78	1.74	0.461
Unknown	0.03	1.03	0.76	1.39	0.871	0.13	1.14	0.82	1.58	0.450
1063: Africa						0.23	1.26	0.10	15.60	0.856
2212: Africa						1.26	3.52	0.29	42.04	0.320
276: Africa						16.83	**20443807.17**	4612284.41	9061654 0.00	**<0.001**
291: Africa						0.23	1.26	0.12	13.34	0.846
420: Africa						-14.61	0.00	0.00	0.00	**<0.001**
449: Africa						1.54	4.69	0.32	67.75	0.257
52: Africa						1.59	4.91	0.19	126.22	0.337
53: Africa						0.23	1.26	0.13	11.88	0.838
542: Africa						0.23	1.26	0.38	4.25	0.706
68: Africa						17.22	**30064422.31**	8521049.85	1060749 00.00	**<0.001**
94: Africa						14.76	**2561880.59**	188487.16	3482058 0.00	**<0.001**
1063: Europe						0.26	1.29	0.86	1.94	0.216
1105: Europe						-0.95	0.39	0.21	0.71	**0.002**
209: Europe						16.42	13494230.27	3736777.96	4873028 0.00	**<0.001**
2212: Europe						-0.29	0.75	0.28	2.05	0.576
251: Europe						0.47	1.60	0.26	9.89	0.611
276: Europe						2.19	**8.96**	1.77	45.42	**0.008**
291: Europe						0.26	1.29	0.81	2.07	0.283
333: Europe						0.26	**1.29**	0.62	2.72	0.500
410: Europe						0.26	1.29	0.57	2.94	0.539
420: Europe						1.09	2.97	0.34	25.68	0.323
449: Europe						0.51	1.66	0.86	3.21	0.132
463: Europe						1.20	3.30	0.88	12.48	0.078
486: Europe						15.25	**4177683.02**	537233.15	3.248689 0.00	**<0.001**
52: Europe						0.13	1.14	0.34	3.90	0.830
53: Europe						0.26	1.29	0.32	5.28	0.720
542: Europe						0.26	1.29	0.81	2.06	0.281
68: Europe						0.87	2.40	0.85	6.73	0.097
75: Europe						15.65	**6231710.49**	2100920.53	1.848438 0.00	**<0.001**
94: Europe						-0.45	0.64	0.04	10.52	0.751

Analysis of Deviance:

Additive Model:

- Formula: Beta ~ UT + Continent
- Residual DF: 7121
- Residual Deviance: 6041.2

Interaction Model:

- Formula: Beta ~ UT * Continent
- Residual DF: 7025
- Residual Deviance: 5842.8

Comparison Between Models:

- DF Difference: 96
- Deviance Difference: 198.46
- p-value: <0.001 (**highly significant**)

Conclusion:

Adding the interaction between Plasmid type and Continent significantly improves model fit.

1063: North America	0.20	1.22	0.64	2.31	0.550
1105: North America	-0.40	0.67	0.28	1.59	0.366
209: North America	-2.90	0.05	0.00	0.64	**0.021**
2212: North America	-0.32	0.73	0.23	2.33	0.592
251: North America	1.78	5.95	0.91	38.65	0.062
276: North America	-0.38	0.68	0.15	3.08	0.618
291: North America	0.20	1.22	0.42	3.49	0.715
333: North America	0.20	1.22	0.56	2.64	0.621
410: North America	0.20	1.22	0.17	8.76	0.846
420: North America	-14.65	0.00	0.00	0.00	**<0.001**
449: North America	0.58	1.79	0.88	3.66	0.109
463: North America	17.66	46890954.41	21570754.29	1.019325e+08	**<0.001**
486: North America	-21.95	**0.00**	0.00	0.00	**<0.001**
52: North America	-1.05	0.35	0.07	1.80	0.210
53: North America	0.20	1.22	0.17	8.87	0.847
542: North America	0.20	1.22	0.72	2.04	0.459
68: North America	-1.38	**0.25**	0.09	0.72	**0.010**
75: North America	15.58	**5861369.32**	1867786.50	1.839378e+00	**<0.001**
94: North America	-2.37	**0.09**	0.01	0.85	**0.035**
1063: Oceania	1.75	5.78	2.53	13.18	**<0.001**
1105: Oceania	-17.78	0.00	0.00	0.00	**<0.001**
2212: Oceania	-0.31	0.73	0.12	4.34	0.731
251: Oceania	-13.03	0.00	0.00	0.00	**<0.001**
276: Oceania	18.35	**93530417.51**	23259879.95	3.760956e+00	**<0.001**
291: Oceania	1.75	5.78	2.24	14.88	**<0.001**
420: Oceania	-13.09	0.00	0.00	0.00	**<0.001**
449: Oceania	-16.60	0.00	0.00	0.00	**<0.001**
463: Oceania	19.22	222691470.92	45642799.54	1.086513e+08	**<0.001**
52: Oceania	-15.45	0.00	0.00	0.00	**<0.001**
542: Oceania	1.75	5.78	1.97	16.92	**0.001**
68: Oceania	3.22	**24.98**	2.85	218.	**0.004**
75: Oceania	17.14	**27836433.77**	7771187.68	9971025.00	**<0.001**
94: Oceania	16.28	**11720603.75**	1148257.33	1.196357e+00	**<0.001**
1063: South America	-0.15	0.86	0.20	3.66	0.837
1105: South America	17.45	37709541.92	11461551.43	1.240678e+08	**<0.001**
209: South America	-1.46	0.23	0.04	1.38	0.109
2212: South America	-19.08	0.00	0.00	0.00	**<0.001**
251: South America	-14.93	0.00	0.00	0.00	**<0.001**
276: South America	-20.68	**0.00**	0.00	0.00	**<0.001**
420: South America	-15.00	0.00	0.00	0.00	**<0.001**
449: South America	2.01	7.44	0.85	65.15	0.070
463: South America	0.83	2.29	0.26	20.40	0.458
52: South America	-1.28	0.28	0.03	2.51	0.254
542: South America	15.05	3425256.96	437858.73	2.679491e+07	**<0.001**
68: South America	-1.73	**0.18**	0.03	1.09	0.061
75: South America	-2.64	**0.07**	0.02	0.33	**<0.001**
94: South America	-1.20	**0.30**	0.02	5.21	0.410
1063: Unknown	-0.13	0.88	0.32	2.45	0.807
1105: Unknown	-0.12	0.89	0.22	3.57	0.871
209: Unknown	-1.15	0.32	0.03	3.00	0.317
2212: Unknown	-0.49	0.61	0.14	2.70	0.518
251: Unknown	1.95	7.04	1.01	48.98	**0.049**
276: Unknown	-0.30	0.74	0.14	3.97	0.724
291: Unknown	-0.13	0.88	0.37	2.08	0.771
333: Unknown	-0.13	0.88	0.11	7.04	0.904
410: Unknown	-0.13	0.88	0.37	2.08	0.772
420: Unknown	-14.97	0.00	0.00	0.00	**<0.001**
449: Unknown	0.42	1.52	0.47	4.99	0.485
463: Unknown	0.85	2.35	0.27	20.18	0.437

486: Unknown	14.86	2843790.27	904428.10	8941720.00	**<0.001**
52: Unknown	-17.34	0.00	0.00	0.00	**<0.001**
53: Unknown	-0.13	0.88	0.41	1.89	0.743
542: Unknown	-0.13	0.88	0.41	1.90	0.745
68: Unknown	-1.48	0.23	0.05	1.00	**0.050**
75: Unknown	15.26	4241987.15	1317255.64	13660560.00	**<0.001**
94: Unknown	-1.77	0.17	0.01	2.97	0.225

➢ C.2. ESBL Models

ESBL Presence

Crude Model
[ESBL ~ Plasmid Type]

Null deviance: **8140.2** on 7146 degrees of freedom
Residual deviance: **5989.4** on 7127 degrees of freedom
AIC: **6029.4**

Plasmid Type	Estimate	OR	CI (low)	CI (high)	P-value
(Intercept)	-1.22	0.29	0.27	0.32	**<0.001**
1063	-17.34	0.00	0.00	0.00	**<0.001**
1105	0.84	2.33	1.80	3.00	**<0.001**
209	-0.81	0.44	0.27	0.72	**0.001**
2212	0.15	1.16	0.76	1.76	0.495
251	-2.67	0.07	0.03	0.17	**<0.001**
276	-0.02	**0.98**	0.70	1.39	0.920
291	-17.34	0.00	0.00	0.00	**<0.001**
333	-17.34	**0.00**	0.00	0.00	**<0.001**
410	-17.34	0.00	0.00	0.00	**<0.001**
420	-2.99	0.05	0.02	0.12	**<0.001**
449	0.83	2.28	1.76	2.97	**<0.001**
463	1.36	3.89	2.73	5.54	**<0.001**
486	3.74	**42.30**	28.52	62.75	**<0.001**
52	-1.27	0.28	0.15	0.52	**<0.001**
53	-17.34	0.00	0.00	0.00	**<0.001**
542	-4.06	0.02	0.00	0.12	**<0.001**
68	2.04	**7.70**	5.65	10.51	**<0.001**
75	1.80	**6.05**	4.42	8.26	**<0.001**
94	1.20	**3.31**	2.39	4.58	**<0.001**

ESBL Presence

Plasmid Type	Additive Model [ESBL ~ Plasmid Type + Continent]					Interaction Model [ESBL ~ Plasmid Type * Continent]				
	Null deviance: **8140.2** on 7146 degrees of freedom Residual deviance: **5951.8** on 7121 degrees of freedom AIC: **6003.8**					Null deviance: **8140.2** on 7146 degrees of freedom Residual deviance: **5685.0** on 7025 degrees of freedom AIC: **5929**				
	Estimate	OR	CI (low)	CI (high)	P-value	Estimate	OR	CI (low)	CI (high)	P-value
(Intercept)	-1.22	0.30	0.27	0.33	**<0.001**	-1.15	0.32	0.28	0.36	**<0.001**
1063	-17.32	0.00	0.00	0.00	**<0.001**	-17.42	0.00	0.00	0.00	**<0.001**
1105	0.84	2.31	1.79	2.99	**<0.001**	0.95	2.58	1.83	3.63	**<0.001**
209	-0.82	0.44	0.27	0.72	**0.001**	-1.09	0.34	0.19	0.60	**<0.001**
2212	0.14	1.14	0.76	1.73	0.523	-0.38	0.69	0.34	1.38	0.29
251	-2.68	0.07	0.03	0.17	**<0.001**	-3.75	0.02	0.00	0.17	**<0.001**
276	-0.05	**0.95**	0.67	1.34	0.776	0.03	**1.03**	0.55	1.90	0.93
291	-17.34	0.00	0.00	0.00	**<0.001**	-17.42	0.00	0.00	0.00	**<0.001**
333*	-17.36	**0.00**	0.00	0.00	**<0.001**	-17.42	**0.00**	0.00	0.00	**<0.001**
410	-17.36	0.00	0.00	0.00	**<0.001**	-17.42	0.00	0.00	0.00	**<0.001**
420	-3.00	0.05	0.02	0.12	**<0.001**	-2.91	0.05	0.02	0.13	**<0.001**
449	0.81	2.25	1.73	2.92	**<0.001**	0.52	1.69	1.15	2.47	0.01
463	1.34	3.83	2.67	5.48	**<0.001**	0.52	1.68	1.01	2.80	0.05
486	3.74	**42.04**	28.11	62.85	**<0.001**	3.77	**43.29**	28.37	66.05	**<0.001**
52	-1.33	0.26	0.14	0.49	**<0.001**	-0.70	0.50	0.21	1.19	0.12
53	-17.36	0.00	0.00	0.00	**<0.001**	-17.42	0.00	0.00	0.00	**<0.001**
542	-4.11	0.02	0.00	0.12	**<0.001**	-17.42	0.00	0.00	0.00	**<0.001**
68	2.09	**8.12**	5.84	11.28	**<0.001**	1.65	**5.19**	3.27	8.24	**<0.001**
75	1.80	**6.07**	4.40	8.37	**<0.001**	2.19	**8.98**	5.72	14.11	**<0.001**
94	1.17	**3.22**	2.31	4.47	**<0.001**	0.51	**1.66**	0.96	2.87	0.07
Africa	0.84	2.32	1.22	4.41	**0.011**	0.18	1.20	0.51	2.81	0.67
Europe	-0.03	0.97	0.84	1.13	0.723	-0.15	0.86	0.71	1.05	0.13
North America	0.08	1.09	0.88	1.34	0.432	-0.05	0.95	0.73	1.24	0.70
Oceania	-0.71	**0.49**	0.37	0.66	**<0.001**	-1.30	**0.27**	0.15	0.49	**<0.001**
South America	-0.08	0.92	0.65	1.31	0.660	-0.05	0.95	0.61	1.49	0.82
Unknown	0.38	1.46	1.12	1.89	**0.004**	0.40	1.49	1.07	2.07	0.02
1063: Africa						-0.18	0.83	0.06	10.78	0.89
2212: Africa						2.73	15.31	1.24	188.73	**0.03**
276: Africa						2.04	**7.67**	0.63	93.28	0.11
291: Africa						-0.18	0.83	0.07	9.32	0.88
420: Africa						-14.69	0.00	0.00	0.00	**<0.001**
449: Africa						1.54	4.67	0.40	55.00	0.22
52: Africa						1.66	5.27	0.20	142.01	0.32
53: Africa						-0.18	0.83	0.09	8.09	0.87
542: Africa						-0.18	0.83	0.26	2.64	0.75
68: Africa						0.70	**2.02**	0.20	20.53	0.55
94: Africa						0.46	**1.58**	0.14	18.46	0.71
1063: Europe						0.15	1.16	0.76	1.78	0.50
1105: Europe						-0.21	0.81	0.45	1.47	0.49
209: Europe						1.69	5.42	0.46	63.70	0.18
2212: Europe						1.31	3.69	1.23	11.09	0.02
251: Europe						0.77	2.16	0.13	35.05	0.59
276: Europe						-0.48	**0.62**	0.27	1.43	0.26
291: Europe						0.15	**1.16**	0.71	1.88	0.55
333: Europe						0.15	**1.16**	0.56	2.39	0.69
410: Europe						0.15	1.16	0.51	2.64	0.73
420: Europe						-14.36	0.00	0.00	0.00	**<0.001**
449: Europe						0.59	1.80	0.92	3.55	0.09
463: Europe						2.48	11.97	3.63	39.45	**<0.001**
486: Europe						16.09	**9761148.02**	1335121.30	71364310.00	**<0.001**
52: Europe						-1.27	0.28	0.03	2.51	0.26
53: Europe						0.15	1.16	0.28	4.76	0.84
542: Europe						0.15	1.16	0.73	1.84	0.53
68: Europe						1.14	**3.14**	1.38	7.13	**0.01**
75: Europe						-4.03	**0.02**	0.00	0.14	**<0.001**
94: Europe						2.13	**8.43**	2.91	24.44	**<0.001**
1063: North America						0.05	1.05	0.54	2.05	0.88
1105: North America						-0.61	0.54	0.22	1.37	0.20
209: North America						1.60	4.93	0.42	57.82	0.20
2212: North America						1.11	3.03	0.86	10.71	0.09
251: North America						2.75	15.70	1.34	184.24	**0.03**
276: North America						1.58	**4.86**	1.44	16.40	**0.01**
291: North America						0.05	1.05	0.37	3.03	0.92

Analysis of Deviance:

Additive Model:

- Formula: Beta ~ UT + Continent
- Residual DF: 7121
- Residual Deviance: 5951.8

Interaction Model:

- Formula: Beta ~ UT * Continent
- Residual DF: 7025
- Residual Deviance: 5685.0

Comparison Between Models:

- DF Difference: 96
- Deviance Difference: 266.86
- p-value: <0.001 (**highly significant**)

Conclusion:

Adding the interaction between Plasmid type and Continent significantly improves model fit.

333: North America	0.05	**1.05**	0.47	2.37	0.90
410: North America	0.05	1.05	0.15	7.62	0.96
420: North America	-14.45	0.00	0.00	0.00	**<0.001**
449: North America	0.50	1.66	0.78	3.50	0.19
463: North America	2.07	7.93	2.00	31.50	**<0.001**
486: North America	-21.13	**0.00**	0.00	0.00	**<0.001**
52: North America	-0.70	0.49	0.09	2.72	0.42
53: North America	0.05	1.05	0.14	7.76	0.96
542: North America	0.05	1.05	0.61	1.81	0.85
68: North America	-0.63	0.53	0.20	1.44	0.21
75: North America	-0.59	0.56	0.18	1.74	0.31
94: North America	1.39	**4.01**	1.51	10.60	**0.05**
1063: Oceania	1.30	3.66	1.56	8.63	**<0.001**
1105: Oceania	-17.07	0.00	0.00	0.00	**<0.001**
2212: Oceania	-15.74	0.00	0.00	0.00	**<0.001**
251: Oceania	-12.37	0.00	0.00	0.00	**<0.001**
276: Oceania	-16.15	**0.00**	0.00	0.00	**<0.001**
291: Oceania	1.30	3.66	1.39	9.64	**0.01**
420: Oceania	-13.20	0.00	0.00	0.00	**<0.001**
449: Oceania	-16.64	0.00	0.00	0.00	**<0.001**
463: Oceania	20.50	7968311422.20	1630762705.41	3893515000.00	**<0.001**
52: Oceania	-15.42	0.00	0.00	0.00	**<0.001**
542: Oceania	1.30	3.66	1.23	10.94	**0.02**
68: Oceania	3.85	**46.78**	5.46	400.51	**<0.001**
75: Oceania	18.82	**1489189881.11**	59693701.08	3715110000.00	**<0.001**
94: Oceania	2.63	**13.93**	1.04	185.83	**0.05**
1063: South America	0.05	1.05	0.25	4.52	0.95
1105: South America	0.25	1.29	0.16	10.52	0.81
209: South America	1.78	5.91	1.28	27.33	**0.02**
2212: South America	-16.99	0.00	0.00	0.00	**<0.001**
251: South America	-13.62	0.00	0.00	0.00	**<0.001**
276: South America	-17.39	**0.00**	0.00	0.00	**<0.001**
420: South America	-14.45	0.00	0.00	0.00	**<0.001**
449: South America	2.62	13.77	1.67	113.7	**0.01**
463: South America	0.46	1.58	0.34	7.41	0.56
52: South America	-0.59	0.56	0.06	5.34	0.6
542: South America	15.25	4196592.36	537099.89	32789780.00	**<0.001**
68: South America	-0.45	**0.64**	0.11	3.63	0.61
75: South America	-0.30	**0.74**	0.23	2.41	0.62
94: South America	-1.56	**0.21**	0.04	1.06	0.06
1063: Unknown	-0.40	0.67	0.24	1.88	0.45
1105: Unknown	-0.01	0.99	0.28	3.48	0.98
209: Unknown	-16.73	0.00	0.00	0.00	**<0.001**
2212: Unknown	0.03	1.03	0.18	5.75	0.97
251: Unknown	2.02	7.51	0.42	132.75	0.17
276: Unknown	-0.57	0.56	0.13	2.36	0.43
291: Unknown	-0.40	0.67	0.29	1.56	0.35
333: Unknown	-0.40	0.67	0.10	4.68	0.69
410: Unknown	-0.40	0.67	0.28	1.60	0.37
420: Unknown	-14.90	0.00	0.00	0.00	**<0.001**
449: Unknown	-0.11	0.90	0.23	3.44	0.87
463: Unknown	0.93	2.53	0.56	11.37	0.23
486: Unknown	15.55	5666040.97	2004330.08	16017330.00	**<0.001**
52: Unknown	-17.12	0.00	0.00	0.00	**<0.001**
53: Unknown	-0.40	0.67	0.31	1.45	0.31
542: Unknown	-0.40	0.67	0.31	1.47	0.32
68: Unknown	-0.67	0.51	0.12	2.16	0.36
75: Unknown	-0.34	0.71	0.20	2.55	0.60
94: Unknown	1.34	3.83	0.90	16.26	0.07

➢ C.3. Carbapenemase Models

Carbapenemase Presence					
Crude Model [Carbapenemase ~ Plasmid Type]					
Null deviance: **7935.9** on 7146 degrees of freedom Residual deviance: **5818.3** on 7127 degrees of freedom AIC: **5858.3**					
Plasmid Type	**Estimate**	**OR**	**CI (low)**	**CI (high)**	**P-value**
(Intercept)	-1.39	0.25	0.23	0.27	**<0.001**
1063	-17.18	0.00	0.00	0.00	**<0.001**
1105	0.63	1.87	1.43	2.45	**<0.001**
209	-1.46	0.23	0.12	0.461	**<0.001**
2212	-0.40	0.67	0.40	1.13	0.132
251	-2.51	0.08	0.03	0.20	**<0.001**
276	2.88	**17.78**	12.27	25.78	**<0.001**
291	-17.18	0.00	0.00	0.00	**<0.001**
333*	19.95	**462770930.73**	358198648.05	597872000.00	**<0.001**
410	-17.18	0.00	0.00	0.00	**<0.001**
420	-2.82	0.06	0.02	0.15	**<0.001**
449	0.36	1.43	1.07	1.91	**0.015**
463	1.83	6.23	4.35	8.93	**<0.001**
486	2.47	**11.88**	9.29	15.18	**<0.001**
52	-1.21	0.30	0.16	0.57	**<0.001**
53	-17.18	0.00	0.00	0.00	**<0.001**
542	-3.89	0.02	0.00	0.15	**<0.001**
68	1.62	**5.06**	3.79	6.74	**<0.001**
75	1.16	**3.19**	2.35	4.34	**<0.001**
94	1.76	**5.81**	4.12	8.19	**<0.001**

Carbapenemase Presence

Plasmid Type	Additive Model [Cararbapenemase ~ Plasmid Type + Continent]					Interaction Model [Carbapenemase ~ Plasmid Type * Continent]				
	Null deviance: **7935.9** on 7146 degrees of freedom Residual deviance: **5765.3** on 7121 degrees of freedom AIC: **5817.3**					Null deviance: **7935.9** on 7146 degrees of freedom Residual deviance: **5377.6** on 7025 degrees of freedom AIC: **5621.6**				
	Estimate	OR	CI (low)	CI (high)	P-value	Estimate	OR	CI (low)	CI (high)	P-value
(Intercept)	-1.56	0.21	0.19	0.24	**<0.001**	-1.50	0.22	0.20	0.25	**<0.001**
1063	-17.22	0.00	0.00	0.00	**<0.001**	-17.07	0.00	0.00	0.00	**<0.001**
1105	0.63	1.88	1.44	2.46	**<0.001**	0.46	1.59	1.08	2.34	**0.019**
209	-1.36	0.26	0.13	0.51	**<0.001**	-1.48	0.23	0.11	0.49	**<0.001**
2212	-0.33	0.72	0.43	1.21	0.210	-1.80	0.17	0.04	0.68	**0.013**
251	-2.51	0.08	0.03	0.20	**<0.001**	-2.70	0.07	0.02	0.27	**<0.001**
276	2.83	**16.88**	11.56	24.63	**<0.001**	2.62	**13.76**	7.37	25.70	**<0.001**
291	-17.16	0.00	0.00	0.00	**<0.001**	-17.07	0.00	0.00	0.00	**<0.001**
333*	20.04	**50513315 5.50**	38646289 0.83	66024322 3.46	**<0.001**	20.07	**51813975 2.44**	37624825 2.86	71354170 0.00	**<0.001**
410	-17.03	0.00	0.00	0.00	**<0.001**	-17.07	0.00	0.00	0.00	**<0.001**
420	-2.70	0.07	0.03	0.16	**<0.001**	-2.56	0.08	0.03	0.19	**<0.001**
449	0.36	1.43	1.07	1.91	**0.015**	0.48	1.62	1.07	2.44	**0.023**
463	1.83	6.23	4.40	8.82	**<0.001**	0.93	2.53	1.54	4.16	**<0.001**
486	2.64	**13.98**	10.79	18.12	**<0.001**	2.63	**13.84**	10.60	18.07	**<0.001**
52	-1.31	0.27	0.14	0.53	**<0.001**	-0.55	0.57	0.22	1.47	0.247
53	-17.03	0.00	0.00	0.00	**<0.001**	-17.07	0.00	0.00	0.00	**<0.001**
542	-4.05	0.02	0.00	0.12	**<0.001**	-17.07	0.00	0.00	0.00	**<0.001**
68	1.67	**5.31**	3.95	7.13	**<0.001**	1.15	**3.17**	2.00	5.04	**<0.001**
75	1.18	**3.24**	2.38	4.41	**<0.001**	0.40	**1.49**	0.94	2.38	0.092
94	1.70	**5.45**	3.78	7.87	**<0.001**	5.54	**255.38**	35.30	1847.66	**<0.001**
Africa	-0.35	0.71	0.37	1.35	0.294	-1.10	0.33	0.09	1.25	0.103
Europe	0.37	1.45	1.23	1.71	**<0.001**	0.21	1.24	0.99	1.54	0.062
North America	0.34	**1.41**	1.13	1.76	**0.002**	0.39	**1.47**	1.13	1.92	**0.004**
Oceania	-0.46	**0.63**	0.46	0.87	**0.005**	-1.09	**0.34**	0.18	0.64	**0.001**
South America	0.61	**1.84**	1.41	2.40	**<0.001**	0.59	**1.80**	1.24	2.61	**0.002**
Unknown	0.49	1.62	1.23	2.15	**0.001**	0.54	1.71	1.18	2.47	**0.004**
1063: Africa						1.10	3.01	0.31	28.85	0.339
2212: Africa						4.80	122.03	8.46	1760.22	**<0.001**
276: Africa						-1.12	**0.33**	0.05	2.29	0.260
291: Africa						1.10	3.01	0.36	25.38	0.310
420: Africa						-13.40	0.00	0.00	0.00	**<0.001**
449: Africa						1.02	2.78	0.16	48.95	0.484
52: Africa						3.16	23.50	0.91	608.82	0.057
53: Africa						1.10	3.01	0.15	62.31	0.475
542: Africa						1.10	3.01	0.56	16.25	0.200
68: Africa						1.04	**2.84**	0.25	31.95	0.399
94: Africa						-2.94	**0.05**	0.00	2.04	0.115
1063: Europe						-0.21	0.81	0.53	1.24	0.328
1105: Europe						0.09	1.10	0.58	2.09	0.777
209: Europe						2.08	7.98	0.63	100.70	0.108
2212: Europe						1.58	4.86	0.82	28.70	0.081
251: Europe						-0.29	0.75	0.07	8.42	0.814
276: Europe						1.43	**4.17**	1.46	11.89	**0.007**
291: Europe						-0.21	0.81	0.50	1.30	0.384
333: Europe						-0.21	**0.81**	0.38	1.71	0.581
410: Europe						-0.21	0.81	0.35	1.86	0.619
420: Europe						-14.71	0.00	0.00	0.00	**<0.001**
449: Europe						-0.58	0.56	0.25	1.26	0.160
463: Europe						2.40	10.98	2.93	41.17	**<0.001**
486: Europe						-19.91	**0.00**	0.00	0.00	**<0.001**
52: Europe						0.09	1.10	0.27	4.54	0.897
53: Europe						-0.21	0.81	0.20	3.31	0.769
542: Europe						-0.21	0.81	0.49	1.33	0.406
68: Europe						1.42	**4.13**	1.90	8.98	**<0.001**
75: Europe						3.29	**26.72**	5.68	125.56	**<0.001**
94: Europe						-5.82	**0.00**	0.00	0.03	**<0.001**
1063: North America						-0.39	0.68	0.36	1.29	0.236
1105: North America						0.57	1.78	0.76	4.16	0.186
209: North America						-15.97	0.00	0.00	0.00	**<0.001**
2212: North America						2.10	8.14	1.34	49.27	**0.023**
251: North America						0.86	2.37	0.20	27.82	0.491
276: North America						-1.64	**0.19**	0.06	0.64	**0.007**
291: North America						-0.39	0.68	0.24	1.95	0.471

Analysis of Deviance:

Additive Model:

- Formula: Beta ~ UT + Continent
- Residual DF: 7121
- Residual Deviance: 5765.3

Interaction Model:

- Formula: Beta ~ UT * Continent
- Residual DF: 7025
- Residual Deviance: 5377.6

Comparison Between Models:

- DF Difference: 96
- Deviance Difference: 387.75
- p-value: <0.001 (**highly significant**)

Conclusion:

Adding the interaction between Plasmid type and Continent significantly improves model fit.

333: North America	-0.39	**0.68**	0.31	1.47	0.324
410: America	-0.39	0.68	0.09	4.90	0.700
420: North America	-14.89	0.00	0.00	0.00	**<0.001**
449: North America	-0.20	0.82	0.38	1.80	0.624
463: North America	18.75	13874622 0.95	65482763 .54	29397830 0.00	**<0.001**
486: North America	-20.08	**0.00**	0.00	0.00	**<0.001**
52: North America	-16.90	0.00	0.00	0.00	**<0.001**
53: North America	-0.39	0.68	0.09	4.99	0.703
542: North America	-0.39	0.68	0.40	1.16	0.157
68: North America	-0.41	0.66	0.25	1.78	0.415
75: North America	3.35	28.48	3.53	229.76	**0.002**
94: North America	-5.68	**0.00**	0.00	00.30	**<0.001**
1063: Oceania	1.09	2.98	1.22	7.31	**0.017**
1105: Oceania	-16.44	0.00	0.00	0.00	**<0.001**
2212: Oceania	2.68	14.64	1.56	137.37	**0.019**
251: Oceania	-13.28	0.00	0.00	0.00	**<0.001**
276: Oceania	18.54	**11225571 1.43**	29537203 .78	42662620 0.00	**<0.001**
291: Oceania	1.09	2.98	1.10	8.10	**0.032**
420: Oceania	-13.41	0.00	0.00	0.00	**<0.001**
449: Oceania	-16.45	0.00	0.00	0.00	**<0.001**
463: Oceania	20.23	61000493 5.63	12323856 4.77	30193960 00.00	**<0.001**
52: Oceania	-15.42	0.00	0.00	0.00	**<0.001**
542: Oceania	1.09	2.98	0.97	9.15	0.056
68: Oceania	2.42	**11.22**	3.30	38.18	**<0.001**
75: Oceania	2.70	**14.91**	2.54	87.46	**0.003**
94: Oceania	-3.64	**0.03**	0.00	0.59	**0.022**
1063: South America	-0.59	0.55	0.13	2.29	0.415
1105: South America	1.55	4.69	0.44	50.45	0.202
209: South America	0.45	1.56	0.16	15.51	0.704
2212: South America	-15.86	0.00	0.00	0.00	**<0.001**
251: South America	-14.96	0.00	0.00	0.00	**<0.001**
276: South America	-20.28	**0.00**	0.00	0.00	**<0.001**
420: South America	-15.09	0.00	0.00	0.00	**<0.001**
449: South America	1.53	4.61	0.81	26.19	0.085
463: South America	2.06	7.85	0.91	67.85	0.061
52: South America	-17.10	0.00	0.00	0.00	**<0.001**
542: South America	14.61	2210885. 88	285039.8 9	17148530 .00	**<0.001**
68: South America	-0.25	**0.78**	0.14	4.40	0.781
75: South America	-18.06	**0.00**	0.00	0.00	**<0.001**
94: South America	-2.84	**0.06**	0.01	0.63	**0.019**
1063: Unknown	-0.54	0.58	0.22	1.57	0.287
1105: Unknown	1.06	2.89	0.76	11.01	0.121
209: Unknown	-16.12	0.00	0.00	0.00	**<0.001**
2212: Unknown	1.66	5.26	0.59	47.00	0.137
251: Unknown	1.18	3.24	0.28	37.79	0.348
276: Unknown	-0.74	0.48	0.13	1.80	0.274
291: Unknown	-0.54	0.58	0.24	1.41	0.232
333: Unknown	-0.54	0.58	0.07	4.65	0.612
410: Unknown	-0.54	0.58	0.24	1.42	0.235
420: Unknown	-15.04	0.00	0.00	0.00	**<0.001**
449: Unknown	-0.62	0.54	0.14	2.02	0.361
463: Unknown	1.29	3.62	0.63	20.74	0.148
486: Unknown	-0.28	0.76	0.08	6.80	0.804
52: Unknown	-17.05	0.00	0.00	0.00	**<0.001**
53: Unknown	-0.54	0.58	0.27	1.25	0.165
542: Unknown	-0.54	0.58	0.27	1.29	0.182
68: Unknown	-0.41	0.66	0.16	2.78	0.572
75: Unknown	2.03	7.60	1.93	29.93	**0.004**
94: Unknown	-6.19	0.00	0.00	0.03	**<0.001**

➢ D. Stratified Models for Asia and Europe for Carbapenemase-Gene Carraige

Carbapenemase Presence by Stratified by Continent											
ASIA [Reference Plasmid Type: "486"]						**Europe** [Reference Plasmid Type: "276*]					
Null deviance: **4202.7** on 3920 degrees of freedom Residual deviance: **2754.0** on 3901 degrees of freedom AIC: **2794**						Null deviance: **1798.2** on 1517 degrees of freedom Residual deviance: **1232.2** on 1498 degrees of freedom AIC: **1272.2**					
Plasmid Type	**Estimate**	**OR**	**CI (low)**	**CI (high)**	**p-value**	**Plasmid Type**	**Estimate**	**OR**	**CI (low)**	**CI (high)**	**p-value**
(Intercept)	1.13	3.09	2.44	3.91	**<0.001**	**(Intercept)**	2.76	15.83	6.93	36.17	**<0.001**
Other	-2.63	0.07	0.06	0.09	**<0.001**	**Other**	-4.05	0.02	0.01	0.04	**<0.001**
1063	-19.69	0.00	0.00	0.00	**<0.001**	**1063**	-21.33	0.00	0.00	0.00	**<0.001**
1105	-2.16	0.11	0.07	0.18	**<0.001**	**1105**	-3.49	0.03	0.01	0.08	**<0.001**
209	-4.11	0.02	0.01	0.04	**<0.001**	**209**	-3.46	0.03	0.00	0.40	**0.008**
2212	-4.42	0.01	0.00	0.05	**<0.001**	**2212**	-4.27	0.01	0.00	0.05	**<0.001**
251	-5.33	0.00	0.00	0.02	**<0.001**	**251**	-7.04	0.00	0.00	0.01	**<0.001**
276	-0.01	0.99	0.52	1.91	0.986	**291**	-21.33	0.00	0.00	0.00	**<0.001**
291	-19.69	0.00	0.00	0.00	**<0.001**	**333***	15.80	7304134.28	2546288.26	20952214.39	**<0.001**
333*	17.44	**37440256.76**	25012161.74	56043649.53	**<0.001**	**410**	-21.33	0.00	0.00	0.00	**<0.001**
410	-19.69	0.00	0.00	0.00	**<0.001**	**420**	-21.33	0.00	0.00	0.00	**<0.001**
420	-5.19	0.01	0.00	0.01	**<0.001**	**449**	-4.15	0.02	0.01	0.04	**<0.001**
449	-2.15	0.12	0.07	0.18	**<0.001**	**463**	-0.73	0.48	0.11	2.09	0.331
463	-1.70	0.18	0.11	0.31	**<0.001**	**486**	-21.33	0.00	0.00	0.00	**<0.001**
52	-3.18	0.04	0.02	0.11	**<0.001**	**52**	-4.51	0.01	0.00	0.04	**<0.001**
53	-19.69	0.00	0.00	0.00	**<0.001**	**53**	-21.33	0.00	0.00	0.00	**<0.001**
542	-19.69	0.00	0.00	0.00	**<0.001**	**542**	-21.33	0.00	0.00	0.00	**<0.001**
68	-1.47	0.23	0.14	0.38	**<0.001**	**68**	-1.48	0.23	0.08	0.65	**0.005**
75	-2.23	0.11	0.06	0.18	**<0.001**	**75**	-0.36	0.69	0.13	3.66	0.667
94	2.92	18.45	2.51	135.40	**0.004**	**94**	-4.33	0.01	0.00	0.05	**<0.001**

D. Intraclass Correlation Coefficient (ICC)

➢ Logistic Mixed Model (Intercept-Only)

A generalized linear mixed model (GLMM) was used to estimate the baseline probability of beta-lactamase gene carriage, with genome (Gembase name) included as a random intercept. The model showed a singular fit with zero variance for the random effect, indicating that plasmid-level beta-lactamase carriage was not meaningfully clustered by genome. This suggests that most of the variation is explained at the plasmid level rather than the genome level.

- Random effect (genome-level intercept): Variance = 0
- Observations: 7,147 plasmids across 1,959 genomes
- Model fit: AIC = 9597.7, log-likelihood = –4796.8

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