Le Infezioni in Medicina, n. 1, 57-70, 2026
doi: 10.53854/liim-3401-6

Genomic epidemiology of Carbapenem-Resistant Enterobacterales in southern Vietnam: dominance of *Klebsiella pneumoniae* ST16 and horizontal gene transfer

Thien Phu Truong, Trong Tin Tran, Phuong Mai Le, Van Thanh Nguyen, Tuan Khanh Ta, Thi Tuyet Tran, Cong Tri Tran, Pham My Da Le, Quang Tin Nguyen, Thi Nam Phuong Nguyen
Microbiology Department, Cho Ray Hospital, Ho Chi Minh City, Vietnam.

Article received 12 November 2025 and accepted 9 January 2026

SUMMARY

Background: Carbapenem-resistant Enterobacterales (CRE) pose a critical global threat. However, the genomic epidemiology, transmission dynamics (clonal vs. horizontal gene transfer), and mechanisms driving co-resistance in Southern Vietnam remain poorly understood. This study aimed to use Whole-Genome Sequencing (WGS) to characterize the molecular epidemiology, transmission mechanisms, and co-resistance patterns of CRE from a major referral center in Southern Vietnam

Methodology: We performed a cross-sectional study using whole-genome sequencing on 189 CRE isolates (*K. pneumoniae*, *E. coli*, *E. cloacae*) from a major referral hospital in Southern Vietnam. We analyzed Carbapenemase-Producing Genes (CPGs), MLST, colistin resistance mutations, plasmid clusters, and co-carried AMR genes.

Results: K. pneumoniae ST16 (n=67, 35.4%) was the most frequently identified clone, detected in 10/12 ward strata. We identified two distinct colistin resistance pathways linked to CPG lineage: bla_{KPC}/bla_{OXA-48} family clones (ST147, ST5815, ST11) showed a universal prevalence of chromosomal *pmrB* mutations (n=55/55, 100%), whereas the bla_{NDM} clone (ST16) exhibited a low frequency of these mutations (6.0%). Analysis of 10 plasmid clusters carrying CPGs revealed the frequent co-carriage of *qnrS1* (quinolone resistance) and *rmtB1* (amikacin resistance).

Conclusions: CRE dissemination in Southern Vietnam is driven by a dual-transmission scenario. We identified distinct CPG-linked colistin resistance pathways and significant co-carriage of *qnrS1* with CPGs. This highlights the potential risk of co-selection through antibiotic pressure. These findings underscore the urgent need for surveillance strategies targeting high-risk clones like *K. pneumoniae* ST16.

Keywords: Carbapenem-resistant Enterobacterales (CRE), Whole-Genome Sequencing (WGS), genomic epidemiology, *Klebsiella pneumoniae* ST16, Colistin resistance.

INTRODUCTION

The emergence and dissemination of multidrug-resistant (MDR) Enterobacterales represent a serious global threat to public health [1–3]. In low and middle-income countries, carbapenems are a common choice for treating MDR Enterobacterales, due to the widespread resistance to third-generation cephalosporins [4]. Consequently, the prevalence of Carbapenem-Resistant Enterobacterales (CRE) isolates has increased dramatically under selective pressure, especially in Vietnam. This trend severely limits therapeutic options and raises concerns about the transmission of carbapenem resistance mechanisms [1, 5-8].

Corresponding author
Thien Phu Truong
E-mail: truongthienphu78@yahoo.com

While the distribution of Carbapenemase-Producing Genes (CPGs) varies globally, the specific genomic profiles and their plasmid-mediated spread remain unclear in Southern Vietnam [9]. Most CRE acquire resistance through CPGs located on plasmids, which facilitate the horizontal transmission of high-level resistance variants in both hospital and community settings [8–14]. However, it is not well understood whether CRE dissemination in our region is primarily driven by clonal expansion or by horizontal gene transfer. Furthermore, these plasmids often act as MDR reservoirs, co-harboring resistance genes such as *rmtB1* (aminoglycoside) [15] and *qnrS1* (quinolone) [16]. Although this co-carriage leads to difficult-to-treat MDR phenotypes, the specific patterns of this co-harboring in local CRE isolates remain poorly described. Additionally, as colistin is a last-resort antibiotic widely used to treat CRE infections, the emergence of resistance to it is a critical concern. Whether different CPG-producing clones have a preference for different colistin resistance mechanisms remains unclear.

This study represents the first genomic epidemiology investigation using Whole-Genome Sequencing (WGS) at Cho Ray Hospital, a major quaternary referral center serving the 57 million inhabitants of Southern Vietnam (approximately 60% of the national population). We aimed to:

1) characterize the molecular epidemiology of CRE to clarify transmission mechanisms (horizontal gene transfer vs. clonal expansion);
2) investigate the association between bacterial lineages and dominant colistin resistance mechanisms;
3) analyze the role of CPG-plasmids as MDR reservoirs to understand co-selection mechanisms.

METHODS AND MATERIALS

Ethics Statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Cho Ray Hospital (Ho Chi Minh City, Vietnam) (*Date*: 05/01/2024, *reference number*: 38/QĐ-BVCR). The need for written informed consent was waived. Clinical trial number: not applicable. Human Ethics and Consent to Participate declarations: not applicable.

Study design and setting

The descriptive, cross-sectional study was conducted at Cho Ray Hospital, Ho Chi Minh City, Vietnam, from February 1 to August 31, 2023. The hospital has an average of 2,500 inpatients and 5,000 outpatients daily. Due to the large number of patients and complex infectious disease cases, Cho Ray Hospital is ideally positioned to serve as a key site for monitoring antimicrobial resistance patterns and developing guidelines for antibiotic therapy. This effort provides valuable data to inform national and regional health policies.

All isolates from diagnostic culture were identified, and Antimicrobial Susceptibility Testing (AST) was conducted using the MALDI-TOF/MS system (bioMérieux, France) and the automated VITEK-2 system (bioMérieux, France). Quality control strains used for identification and AST included *Escherichia coli* ATCC 8739, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. AST results were interpreted based on the breakpoints specified in the Clinical & Laboratory Standards Institute (CLSI) M100 guideline (34th edition) [17].

To collect isolates for the study, we followed these inclusion criteria:

1) identified as *E. coli*, *K. pneumoniae*, or *E. cloacae* complex;
2) CRE (resistant or intermediate to at least one carbapenem: ertapenem, imipenem, or meropenem);
3) non-duplicate isolates (defined as one isolate per patient). We used a quota sampling strategy, which ensured genomic diversity across 12 strata with a target of 18 isolates per stratum (3 ward groups [Surgery, Internal Medicine, and Intensive Care Units] x 4 specimen groups [Blood, Sputum, Urine, Others]). CRE isolates were collected consecutively until the quota was met or the study period ended.

DNA extraction and whole genome sequencing

DNA extraction followed the manufacturer's instructions, using the DNEASY® Blood & Tissue kit (Qiagen, Germany). Library preparation used the NEBNEXT® ULTRA™ II FS DNA Library Prep Kit for Illumina (New England Biolabs, the U.S.). Following the library preparation process, paired-end short-read sequencing was conducted using the MiSeq Reagent Kit v3 (2×75 bp) (Illumina, USA) on the MiSeq system (Illumina, USA).

Bioinformatics analysis

The sequence data were analyzed initially on Terra.bio platform (https://app.terra.bio) using TheiaProk_Illumina_PE (version 2.0.1) workflow, a standardized computational pipeline recognized by the U.S. Centers for Disease Control and Prevention for bacterial genomics, available on the website dockstore.org [18]. The workflow is described in *Figure 1*. The bioinformatics tools included: fastq-scan version 0.4.4 [19], trimmomatic version 0.39 (PE, SLIDINGWINDOW:10:20, MINLEN:36) [20], Shovill version 1.1.0 (assembler: SKESA) [21], GAMBIT version 0.5.0 [22], MLST by Torsten Seemann version 2.23.0 [23]. De novo assembly process assessed by QUAST version 5.0.2, with N50 ≥ 20,000 bp and number of contigs ≤ 500 considered as minimal quality for downstream analysis [24]. CG-pipeline was used to estimate coverage depth with a minimal standard of 20x [25]. The fasta files were subsequently downloaded for further analysis. Quality assessment results are available from the corresponding author upon request.

Phylogenetic tree analysis

For phylogenetic analysis, Roary version 3.13.0 [26] (the default 95% BLASTp identity cutoff) was used to calculate the pan-genome from GFF3 files produced by Bakta [27]. The resulting core genome alignment was analyzed with Gubbins (v2.4.1) to infer the phylogenetic tree [28]. This tree was visualized using phandango.net [29].

Acquired antimicrobial resistance (AMR) profile and plasmid cluster analysis

We employed MOB-suite version 3.1.4 for typing and reconstruction of plasmid sequences from WGS assemblies using the default database [30]. Since plasmid sequences were reconstructed from short-read data, they are technically predicted structures. However, for brevity, we refer to them as "plasmids" throughout the text. AMRFinderPlus version 3.12 (database 2024-05-02.2) was used to identify acquired AMR genes [31]. Plasmids carrying CPGs separated by MOB-recon were subjected to pairwise alignment using FastANI (options: – fragLen 200 – minFraction 0.5 [32]) with default Average Nucleotide Identity (ANI) parameter (80%) [33]. Clusters were defined using thresholds of ANI ≥99% and bidirectional alignment coverage (COV) ≥90%, and required to be present in at least two different sequence types (STs) or species. The COV was calculated by dividing the number of passed fragments (FastANI output column 4) by the total fragments in the reference plasmid (FastANI

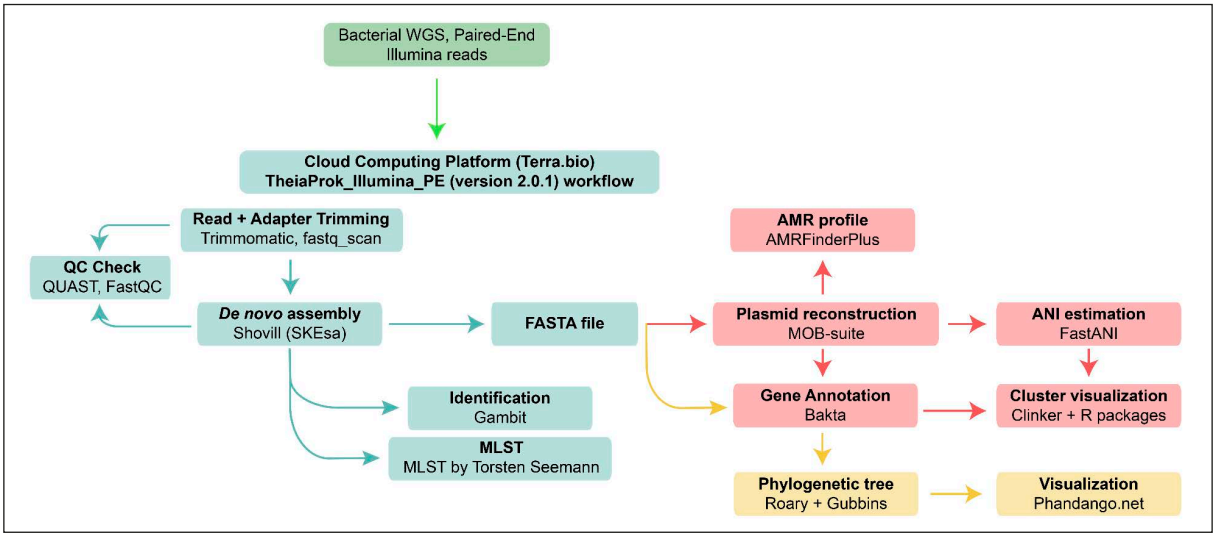

Figure 1 - Bioinformatics analysis: TheiaProk_Illumina_PE (version 2.0.1) workflow on Terra.bio combined with local computational analysis. Raw sequencing data were quality-checked using FastQC and CG-Pipeline, and low-quality sequences and adapters were removed using Trimmomatic and BBDuk. Then, Shovill performed de novo assembly (option: Skesa), followed by downstream analysis using other tools shown in the figure.

output column 5). The plasmid clusters were visualized as an ANI-based network using R packages (igraph, ggraph, tidyverse) and the genetic organization of CPGs in plasmid clusters using Clinker [34].

Data preprocessing before final analysis
Following bioinformatic analyses, isolates that did not find any genes known as carbapenem resistance were subjected to confirmatory AST using disk diffusion with carbapenems (ertapenem (10µg), imipenem (10µg), and meropenem (10µg)) following CLSI M02 guideline [35]. The isolates that were susceptible to all carbapenems were excluded from the final analysis.

Statistical analysis
All statistical analyses, including description and comparison, were conducted using Microsoft Excel 2019 (Microsoft Corporation, Redmond, Washington, USA) and R analysis software version 4.3.0 (R Development Core Team), using the $\chi 2$ statistical test or Fisher's exact test. A p-value of ≤ 0.05 indicated statistical significance. Sankey charts were created using SankeyMatic (https://sankeymatic.com), a web-based tool developed by Steve Bogart.
All processes were performed at the Microbiology Department of Cho Ray Hospital, which is certified under ISO 15189.

RESULTS

A total of 201 CRE isolates were collected. After confirming and removing 7 isolates based on disk diffusion results and removing 5 isolates with low-quality sequence data, 189 isolates were included in the formal analysis. The demographic characteristics of 189 patients identified with various infectious diseases are described in *Table 1*.

Frequency of carbapenemase genes and ST across CRE isolates
Our collection of 189 CRE isolates comprised *K. pneumoniae* (n=149), *E. coli* (n=33), and *E. cloacae* complex (n=7). Among *K. pneumoniae*, ST16 was the most frequently identified clone (n=67). Notably, despite our quota sampling strategy, which ensured diversity by sampling across 12 strata, ST16 demonstrated pervasive dominance. It was the sole most predominant clone in 9 of the 12 strata, and jointly predominant in one other stratum.

Table 1 - Demographic characteristics of patients infected with Carbapenem-Resistant Enterobacterales.

	Number of patients (N=189)
Age: 15 – 94 years old (Median: 61 [IQR, 45 to 71])	
Gender	
Male	115
Female	75
Health conditions	
Underlying diseases/ major interventions (Patients may have one or more conditions)	*167*
Kidney disease	42
Surgical operation	36
Cerebrovascular disease	24
Diabetes	24
Cardiology disease	24
Cancer	23
Haematology disease	9
Severe Burn	8
Biliary tract disease	6
Liver disease	4
Kidney transplantation	3
Others	20
No information	23
Treating Department	
Intensive Care Unit	57
Internal Medicine	68
Surgery	65
Type of infection	
Bloodstream	50
Respiratory	51
Urinary tract	42
Others (Skin, soft tissue, abdominal infections, meningitis, conjunctivitis)	47

As shown in *Table 2*, the distribution of ST16 was similar across the three ward groups (p = 0.188), suggesting that this clone is well-adapted to various hospital environments rather than restricted to specific units. Crucially, this ability allowed ST16 to establish a clear lead (n=67), representing a prevalence more than 2.5-fold higher than the

Table 2 - Distribution of major sequence types across clinical departments and specimen categories.

Characteristics	*ST16 (n=67)*	*ST5815 (n=25)*	*ST147 (n=19)*	*Other STs (n=38)*
Department				
p value[a]	0.188	0.188	0.207	–
ICU	23 (34.3%)	7 (28.0%)	9 (47.4%)	14 (36.8%)
Internal Medicine	26 (38.8%)	5 (20.0%)	7 (36.8%)	8 (21.1%)
Surgery	18 (26.9%)	13 (52.0%)	3 (15.8%)	16 (42.1%)
Specimen Type				
p value[a]	0.029	0.029	0.029	–
Blood	20 (29.9%)	3 (12.0%)	4 (21.1%)	8 (21.1%)
Sputum/BAL	*12 (17.9%)**	*14 (56.0%)**	*10 (52.6%)**	9 (23.7%)
Urine	16 (23.9%)	4 (16.0%)	0 (0.0%)	10 (26.3%)
Others	19 (28.4%)	4 (16.0%)	5 (26.3%)	11 (28.9%)

[a] *p*-values across departments (3 categories) and specimen types (4 categories) were calculated using the Chi-square or Fisher's exact test, comparing the distribution of each clone against the rest of the population (One-vs-Rest, 2x3 or 2x4) and adjusted using the Benjamini-Hochberg method
*Global 2x4 tests indicated a non-uniform distribution across specimen types ($p < 0.05$). Follow-up 2x2 exploratory comparisons revealed significantly higher frequencies of ST5815 ($p = 0.008$) and ST147 ($p = 0.044$) in sputum/BAL, and a significantly lower frequency of ST16 ($p = 0.008$), interpreted within the constraints of the quota-based sampling design.

second most common lineage (ST5815, n=25). In contrast, there was a significant variation in the pattern of three major clones across specimen types ($p < 0.05$). Notably, this was primarily driven by ST5815 and ST147 in respiratory samples ($p < 0.05$). In sputum, these clones were significantly more prevalent, whereas the overall dominant ST16 showed a lower relative abundance compared to its baseline ($p = 0.008$). Among *E. coli*, ST410 (n=13) was dominant. The full distribution of all STs followed by quota sampling is detailed in *Supplementary Table S1*.

As detailed in *Figure 2*, CPGs, including bla_{KPC-2}, bla_{NDM}, bla_{OXA-48} family, were detected in 97.4%

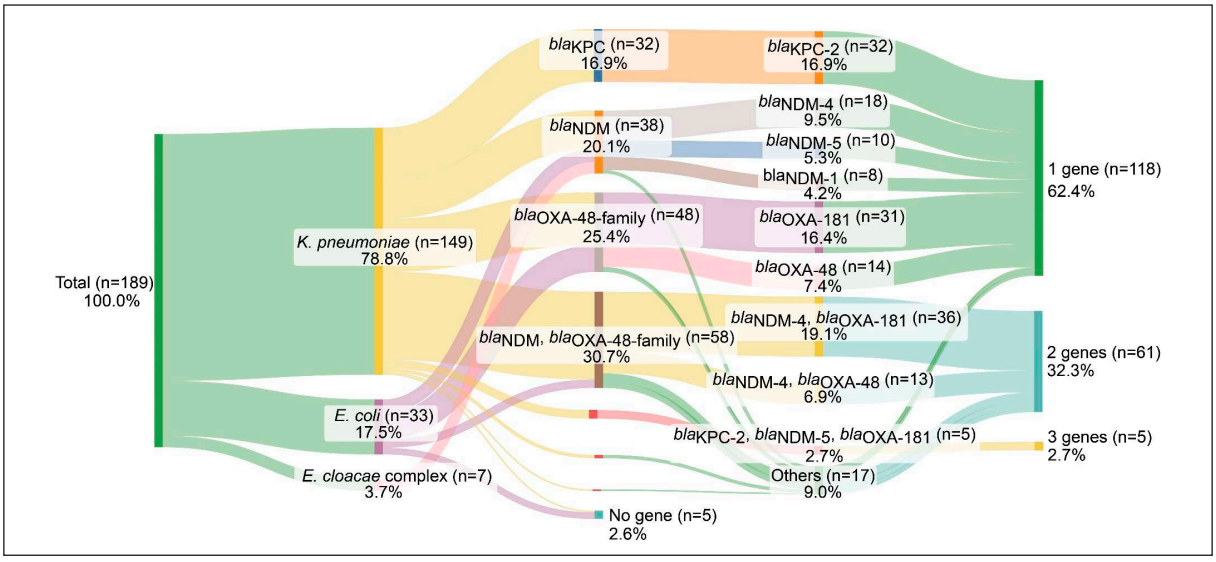

Figure 2 - Distinct carbapenemase genotype profiles among *K. pneumoniae*, *E. coli*, and *E. cloacae* complex. The diagram visualizes the complexity of resistance profiles within the sequenced collection (n=189). The width of the flows corresponds to the number of isolates in this study and does not reflect hospital-wide clinical prevalence. *K. pneumoniae* exhibits the highest diversity and rate of multiple gene co-carriage.

CRE isolates, which were predicted to be carbapenemase-producing Enterobacterales. A significant difference in the number of CPGs per isolate was observed across species ($p<0.001$). All *E. cloacae* complex isolates carried a single CPG, while 40.9% *K. pneumoniae* isolates co-harbored multiple CPGs, compared to 15.2% *E. coli* isolates. In *K. pneumoniae*, the most frequent genotype was the co-harboring of bla_{NDM} and bla_{OXA-48} family, specifically bla_{NDM-4} and $bla_{OXA-181}$. In contrast, in *E. coli*, the bla_{OXA-48} family was most common. Among the *E. cloacae* complex isolates, bla_{NDM-1} was exclusively detected. bla_{KPC-2} was found only in *K. pneumoniae*.

Phylogenetic analysis and association of STs of E. coli and K. pneumoniae isolates with carbapenemase genotypes

Of the 149 *K. pneumoniae* isolates analyzed, one isolate (KLPN_054) was excluded from the phylogenetic analysis. A preliminary pairwise single nucleotide polymorphism (SNP) analysis confirmed this isolate was a highly divergent outlier, differing from the main *K. pneumoniae* population by approximately 40,000 SNPs. This isolate was removed by Gubbins to improve the resolution of the core-genome tree. The final core-genome SNP-based phylogenetic tree of the remaining 148 isolates is presented in *Figure 3A*. A strong association was observed between STs and carbapenemase-producing genotypes in *K. pneumoniae*. The predominant ST16 lineage (n=67) was distinguished by the high proportion of co-harboring bla_{NDM} and bla_{OXA-48} family (71.6%). In contrast, other high-risk clones were largely restricted to single-CPG profiles; specifically, ST5815 (n=25) and ST11 (n=11) predominantly harbored bla_{KPC-2} (92.0% and 63.6%, respectively), while bla_{OXA-48} family was the main genotype in ST147 (n=19; 63.2%). The minor clone ST656 (n=8) exhibited a distinct co-harboring profile of bla_{KPC-2}, bla_{NDM-5}, and $bla_{OXA-181}$ (in 5 isolates). Similarly, a core-genome SNP-based phylogenetic tree was constructed for the 33 *E. coli* isolates (*Figure 3B*). This analysis confirmed that a diversity of genotypes was observed in *E. coli* ST410, whereas *E. coli* ST405 (n=6) was associated with bla_{OXA-48} family (in 5 isolates).

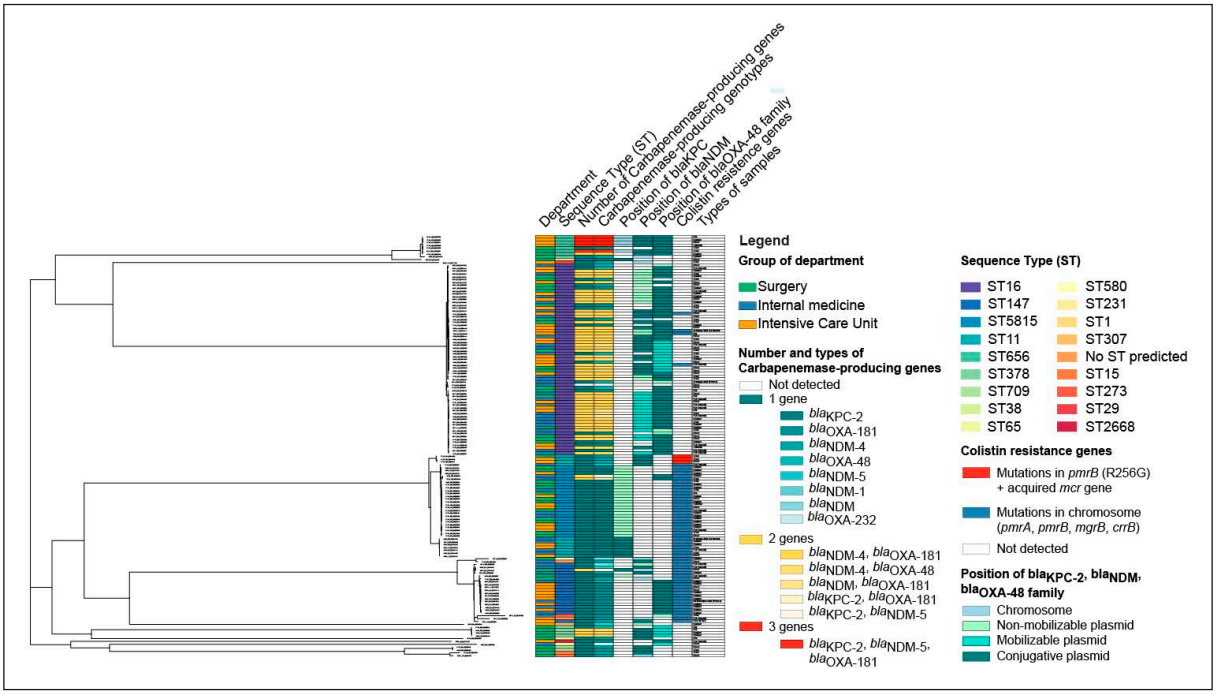

Figure 3A - Phylogenetic construction of 148 Carbapenem-resistant *K. pneumoniae*. The columns represent related data for these isolates, including source, sequence type (ST), genotype, carbapenemase-producing gene positions in the genome, and colistin resistance genes.

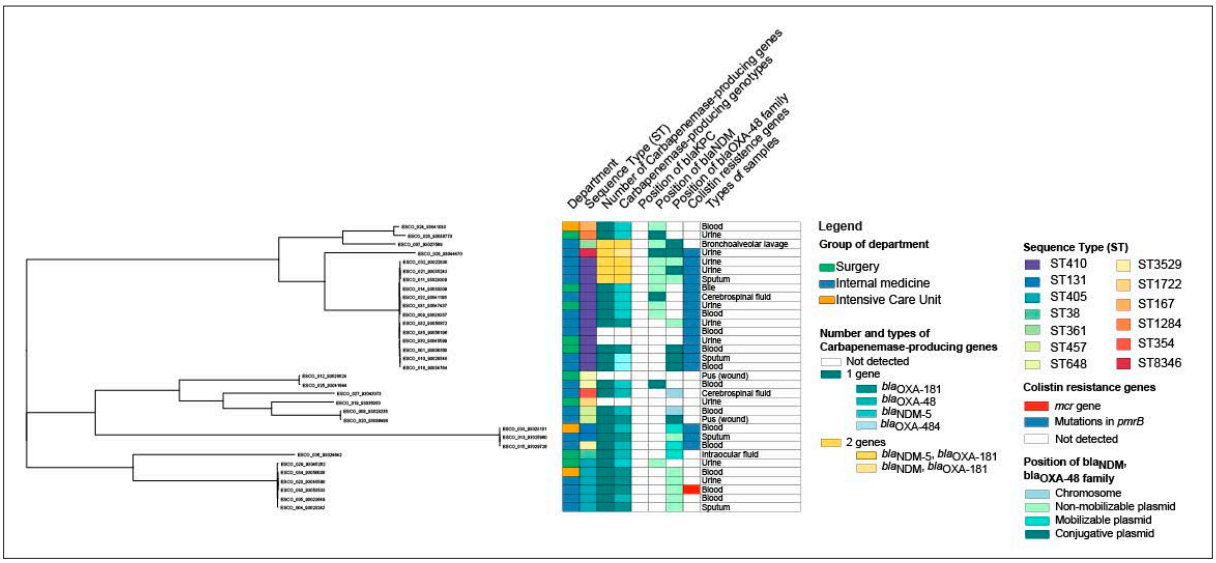

Figure 3B - Phylogenetic construction of 33 Carbapenem-resistant E. coli. The columns represent related data for these isolates, including source, sequence type (ST), genotype, carbapenemase-producing gene positions in the genome, and colistin resistance gene.

Genetic mechanism for Colistin resistance

Genomic analysis identified known colistin resistance determinants in 81 of our 189 isolates. Chromosomal mutations were the predominant genetic mechanism, detected in 98.8% of these isolates. In contrast, plasmid-mediated *mcr* genes were found in only 4.9% of isolates. Notably, three of these *mcr*-positive isolates also harbored a chromosomal mutation (*pmrB*_R256G). Of the 80 isolates with chromosomal mutations, the vast majority (96.3%) had mutations in *pmrB*, with R256G (n=56) being the most common variant. A comprehensive list of all detected colistin resistance genotypes, including chromosomal and plasmid-mediated, across all STs is provided in *Supplementary Table S2*. These findings indicate that chromosomal mutations constitute the primary genetic basis for colistin resistance. In *K. pneumoniae* ST5815, ST11, and ST147, which typically carry bla_{KPC-2} or bla_{OXA-48} family, mutations in *pmrA/pmrB* genes were found in 100% of isolates. In contrast, isolates belonging to *K. pneumoniae* ST16 and ST656, which usually harbor bla_{NDM} with or without other CPG groups, remained largely wild-type, with resistance determinants observed in only 6.0% (4/67) and 0% (0/8), respectively (*Figure 3A*).

Dissemination of plasmids carrying CPG among K. pneumoniae, E. coli, and E. cloacae isolates

A total of 1320 plasmids were found in 189 CRE isolates, of which 234 carried CPGs, detected in 175 of the 189 analyzed isolates. *Table 3* shows that pre-

Table 3 - Structural and replicon profiles of plasmids harboring key carbapenemase families.

Carbapenemase gene	*Total plasmids (n)*	*Plasmid classification as Conjugative (n,%)*	*Major replicon type (%)*	*Predominant variant (n,%)*
bla_{OXA-48} family	105	72 (68.6%)	IncX3 (55.2%) Col156 (18.1%)	$bla_{OXA-181}$ (n=77, 73.3%), mainly in IncX3 bla_{OXA-48} (n=25, 23.8%), mainly in Col156
bla_{NDM}	88	39 (44.3%)	IncFII (47.7%) IncFIB (17.0%)	bla_{NDM-4} (n=57, 64.8%)
bla_{KPC-2}	35	9 (25.7%)	IncU (60.0%)	bla_{KPC-2} (n=35, 100%)
Co-harboring Multi-CPGs	6	5 (83.3%)	Various	bla_{NDM} + bla_{OXA-48} family

dicted dissemination patterns varied significantly by gene family. The bla_{OXA-48} family (predominantly $bla_{OXA-181}$) showed the highest predicted potential for horizontal transmission, with 68.6% located on conjugative plasmids, primarily associated with IncX3 replicons (55.2%). In contrast, bla_{KPC-2} was predicted to have limited plasmid-mediated mobility, being largely confined to IncU plasmids (60.0%) with a low conjugation proportion of 25.7%. bla_{NDM} variants exhibited an intermediate pattern (44.3% conjugative), frequently carried by IncFII plasmids (47.7%). A comprehensive list of all CPG variants, rare replicon types, and their associated mobility is available from the corresponding author upon reasonable request.

Using high-confidence clustering parameters (ANI ≥99%, COV ≥90%), ten plasmid clusters, comprising 43.4% of all CPG-carrying plasmids, were observed in 50.3% of isolates. Notably, the prevalence of these clusters was markedly higher in multiple-CPG isolates compared to single-CPG isolates (91.8% vs. 33.1%). Distinct replicon-gene associations were observed. IncX3, IncFII (Clusters 6, 7, 10), and Col156 (Clusters 4 & 5) were identified as the primary vectors for $bla_{OXA-181}$ and bla_{OXA-48}, respectively. In addition to $bla_{OXA-181}$, IncFII also served as the primary vector for bla_{NDM-1}/bla_{NDM-4} (Clusters 2 & 3). Structural analysis of the genetic context (*Figure 4*) indicated that the annotated genes surrounding bla_{NDM} in clusters 1, 2, and 3 exhibited high synteny, whereas plasmids carrying bla_{OXA-48} family (n=7) were divided into markedly different motifs between bla_{OXA-48} and $bla_{OXA-181}$. Notably, *K. pneumoniae* ST16 was found to carry plasmids from multiple distinct bla_{OXA-48}/$bla_{OXA-181}$ clusters (Clusters 4, 6, 7, 8, and 10), highlighting its role as a major reservoir for these plasmids. In terms of mobility, the vast majority of plasmids in clusters (n=99) were predicted to be conjugative (70.7%) or mobilizable (22.2%), with a minority non-mobilizable (7.1%). Consistent with this, MOBF, MOBP, and MOBQ relaxase types were

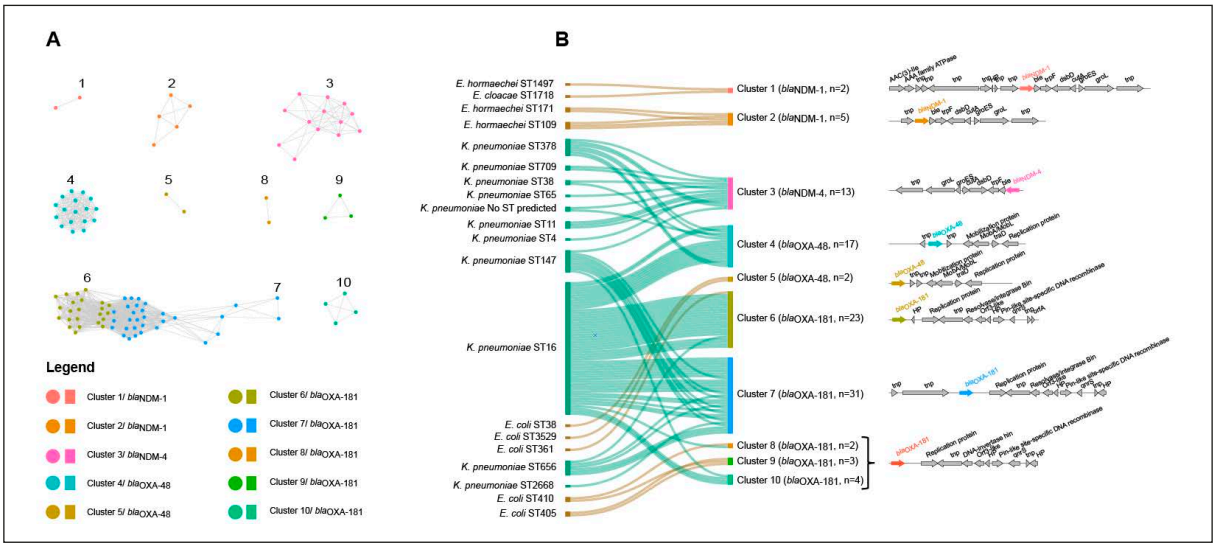

Figure 4- Characterization and correlation of plasmid clusters carrying carbapenemase-producing genes (CPGs) in CRE isolates. (A) Similarity network of 102 plasmids harboring bla_{NDM-1}, bla_{NDM-4}, bla_{OXA-48}, and $bla_{OXA-181}$, grouped into clusters across different STs and species. Clusters were defined using Average Nucleotide Identity (ANI) ≥99.0% and alignment coverage ≥90.0% (calculated with fastANI on plasmid FASTA files from MOB-recon). Each node represents a plasmid and is colored according to its cluster. (B) Distribution of plasmid clusters, showing the number of isolates per cluster across different species and their sequence types (STs). The most frequent genetic organization of CPGs in plasmid clusters, as identified by Clinker. Gene annotations from Bakta are shown: *aac(3)-IIe*, aminoglycoside N-acetyltransferase AAC(3)-IIe; *ble*, bleomycin-binding protein Ble-MBL; *cutA*, divalent-cation tolerance protein CutA; *dsbD*, disulfide interchange protein DsbD; *groES*, co-chaperone GroES; *groL*, chaperonin GroEL; HP, hypothetical protein; *lysR*, LysR family transcriptional regulator; *mobA/mobL*, MobA/MobL domain-containing protein; *orf3*-like, plasmid pRiA4b Orf3-like domain-containing protein; *orfA*, putative insertion sequence 2 OrfA protein; *qnrS*, QnrS family quinolone resistance pentapeptide repeat protein; tnp, transposable element; *traD*, conjugal transfer protein TraD; *trpF*, phosphoribosylanthranilate isomerase.

found in 7 of the 10 clusters, while three clusters carried no relaxase (*Supplementary Table S2*).
Analysis of co-carried AMR genes on the 99 plasmids within the 10 predicted clusters illustrated three key features (*Table 4*). Firstly, a 100% association was found between *qnrS1* and all plasmids within six clusters (Clusters 3, 6, 7, 8, 9, and 10), which primarily carry bla_{NDM-4} or $bla_{OXA-181}$. Secondly, all plasmids in Cluster 2 (bla_{NDM-1}) co-carried *rmtB1*, a 16S rRNA methyltransferase gene known

Table 4 - Frequency of co-harbored antimicrobial resistance genes in 10 clusters of plasmids carrying carbapenemase-producing gene.

AMR genes	*Cluster of plasmids carrying carbapenemase-producing gene*									
	1 (n=2) bla_{NDM-1}	*2 (n=4) bla_{NDM-1}*	*3 (n=12) bla_{NDM-4}*	*4 (n=16) bla_{OXA-48}*	*5 (n=2) bla_{OXA-48}*	*6 (n=23) $bla_{OXA-181}$*	*7 (n=31) $bla_{OXA-181}$*	*8 (n=2) $bla_{OXA-181}$*	*9 (n=3) $bla_{OXA-181}$*	*10 (n=4) $bla_{OXA-181}$*
Quinolone										
qnrS1	–	–	12	–	–	23	31	2	3	4
qnrB6	–	–	–	–	–	–	–	–	–	3
aac(6′)-Ib-cr5	–	–	–	–	–	–	–	–	–	4
High-level Aminoglycoside										
*rmtB*1	–	4	–	–	–	–	–	–	–	–
Other Aminoglycoside										
*aac(3)-II*d	–	–	12	–	–	–	–	–	–	–
aac(3)-IIe	2	–	–	–	–	–	–	–	–	–
aac(6′)-Ib	–	–	7	–	–	–	–	–	–	–
*aadA*1	–	–	10	–	–	–	–	–	–	–
aadA16	–	–	–	–	–	–	–	–	–	2
ESBLs										
$bla_{CTX-M-14}$	–	–	7	–	–	–	–	–	–	–
$bla_{CTX-M-15}$	–	–	–	–	–	–	–	–	–	3
Other beta-lactamases										
bla_{LAP-2}	–	–	11	–	–	–	–	–	–	–
bla_{OXA}	–	–	12	–	–	–	–	–	–	–
bla_{TEM}	–	–	–	–	–	–	–	–	–	1
bla_{TEM-1}	–	2	–	–	–	–	–	–	–	1
Others										
arr-3	–	–	–	–	–	–	–	–	–	4
catA2	–	–	–	–	–	–	–	–	–	1
dfrA27	–	–	–	–	–	–	–	–	–	4
floR	–	–	1	–	–	–	–	–	–	–
mph(A)	–	–	–	–	–	–	–	–	–	4
sul1	–	–	–	–	–	–	–	–	–	4
tet(A)	–	–	–	–	–	–	–	–	–	3

Abbreviation: AMR, antimicrobial resistance; bla_{NDM}, New Delhi Metallo-beta-lactamase; bla_{OXA}, Oxacillinase; ESBL, Extended-Spectrum beta-Lactamase; bla_{CTX-M}, Cefotaximase; *qnrS1*, QnrS family quinolone resistance pentapeptide repeat protein; *qnrB6*, QnrB family quinolone resistance pentapeptide repeat protein; *aac(6′)-Ib-cr5*, Aminoglycoside acetyltransferase (confers resistance to aminoglycosides and low-level quinolones); *rmtB1*, 16S-RMT (Ribosomal Methyltransferase, confers high-level aminoglycoside resistance); *aac(3)-IId / aac(6′)-Ib*, aminoglycoside N-acetyltransferase; *aadA1*, Aminoglycoside nucleotidyltransferase; *arr-3*, NAD(+)--rifampin ADP-ribosyltransferase; *dfrA27*, Dihydrofolate reductase; *sul1*, sulfonamide-resistant dihydropteroate synthase; *floR*, chloramphenicol/florfenicol efflux MFS transporter; *mph(A)*, Macrolide phosphotransferase; bla_{TEM-1}, TEM family class A beta-lactamase; bla_{LAP-2}, class A beta-lactamase LAP-2; *catA2*, Chloramphenicol acetyltransferase; *tet(A)*, tetracycline efflux MFS transporter Tet(A); - indicates the absence of the gene (zero occurrences).

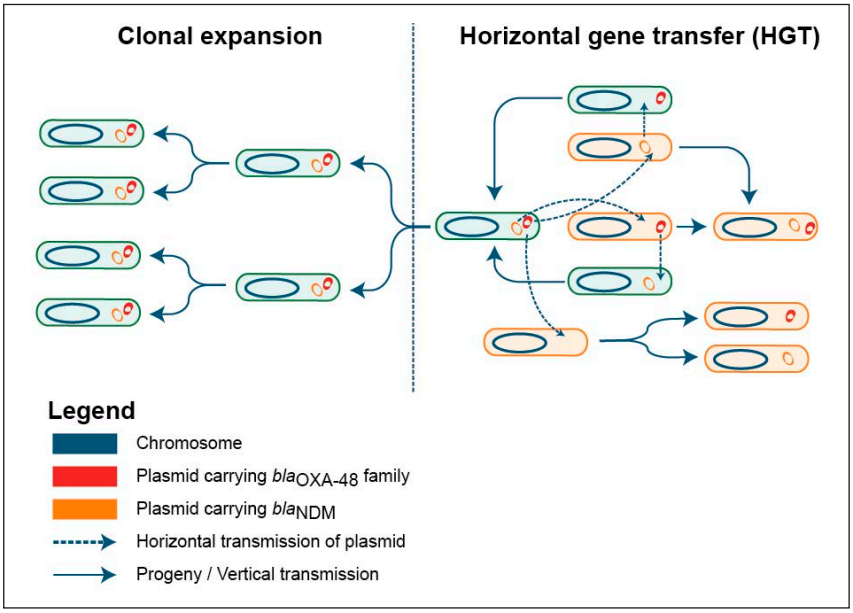

Figure 5
Schematic representation of the dual-transmission dynamics driving CRE dissemination. The diagram illustrates the two concurrent mechanisms contributing to the spread of carbapenem resistance observed in this study.

to confer high-level resistance to most aminoglycosides, including Amikacin. Thirdly, two clusters were identified as major MDR reservoirs. Plasmids in cluster 3 (bla_{NDM-4}) frequently co-carried genes for Extended-spectrum beta-lactamases (ESBLs) ($bla_{CTX-M-14}$) and multiple aminoglycoside resistance genes (*aac(3)-IId*, *aac(6')-Ib*, *aadA1*). Similarly, Cluster 10 ($bla_{OXA-181}$) co-carried a cocktail of genes resisting Quinolones (*qnrS1*, *qnrB6*, *aac(6')-Ib-cr5*), Rifampicin (*arr-3*), Trimethoprim/Sulfonamide (*dfrA27*, *sul1*), and ESBLs ($bla_{CTX-M-15}$), with the majority of plasmids carrying these genes. Collectively, the integration of these clonal and plasmid analyses supports a dual-transmission model driving CRE dissemination, characterized by the interplay between clonal expansion and horizontal gene transfer (*Figure 5*).

DISCUSSION

The present study utilizes whole-genome sequencing analysis to describe the genomic attributes of 189 CRE isolates from Cho Ray Hospital, a major referral center in Southern Vietnam. Our findings presented a complex resistance landscape defined by four key features. First, we observed that *K. pneumoniae* ST16 demonstrated pervasive dominance. This clone was identified as a key MDR reservoir, characterized by its high rate of co-harboring bla_{NDM} and bla_{OXA-48} family. Second, we identified two genomic mechanisms of colistin resistance in *K. pneumoniae* strongly linked to carbapenemase-producing genotypes: bla_{KPC-2} or bla_{OXA-48} family clones (ST5815, ST11, ST147) harbored chromosomal mutations, whereas bla_{NDM} clone (ST16, ST656) lacked these mutations. Third, a dual-transmission of CPG was observed, involving the clonal expansion of high-risk clones (*K. pneumoniae* ST16 and ST5815) and horizontal gene transfer, supported by 10 plasmid clusters found across different species and STs. Finally, we noted the high prevalence of co-harboring other critical AMR genes (such as *qnrS1* and *rmtB1*) within the clusters of plasmids carrying CPG.

The prevalence of *K. pneumoniae* ST16 in Asia has been rising, along with an increasing number of acquired AMR genes in its genome, since its initial report in 2003 [36, 37]. Consistent with this regional trend, our study confirms the pervasive dominance of the ST16 lineage in Southern Vietnam. This finding mirrors recent reports from Northern Vietnam [38], marking a clear epidemiological shift from the previously reported ST15 lineage [39]. Regionally, this trend is not unique to Vietnam; in Thailand, *K. pneumoniae* ST16 was also observed to frequently co-harbor two CPGs, specifically bla_{NDM-1} and $bla_{OXA-232}$ [37]. Although ST16 is not considered a hypervirulent clone, its heavy resistance burden

has been associated with severe infections and higher mortality rates compared to the high-risk clonal complex 258, which includes the widely reported ST258, ST11, and ST437 [40, 41].

K. pneumoniae ST5815 carrying bla_{KPC-2} represents a unique lineage circulating in southern Vietnam, possibly reflecting local clonal expansion. Interestingly, unlike the ubiquitous ST16 found across various specimen types, this ST5815 lineage showed a significant predilection for respiratory samples, hinting at a specific niche adaptation. Furthermore, we observed a divergence from global trends regarding high-risk clones. While *K. pneumoniae* ST11 and ST147 were typically associated with the global dispersion of bla_{OXA-48} [42], our study identified that ST11 was linked to bla_{NDM} or bla_{KPC-2}, whereas bla_{OXA-48} family was still mainly found in ST147. In contrast, the Carbapenem-resistant *Escherichia coli* population was less complex, dominated by the globally prevalent lineages ST410 and ST405 [43].

While in East and Southeast Asia, the most prevalent CRE isolates carried a single CPG, including bla_{KPC} in the Republic of Korea, Singapore, and China, bla_{NDM} in the Philippines, bla_{IMP} in Japan [11, 12, 44-46], our study showed that a remarkably high proportion of isolates, co-harboring bla_{NDM} and bla_{OXA-48} family, was detected (n=58, 30.7%). Although this finding is notably different from other reports globally, it is consistent with recent data reported from Northern Vietnam, suggesting that this represents a shared clinical challenge across Vietnamese regions [38, 47]. Our data indicate that this co-harboring genotype is driven by two main factors. Firstly, the clonal expansion of the *K. pneumoniae* ST16 lineage accounted for approximately 80% of these multi-CPG isolates. Secondly, horizontal gene transfer contributed to approximately 10% of the cases (specifically among non-ST16 isolates), as supported by their presence in predicted clusters of plasmids carrying CPGs.

The co-harboring genotype has direct clinical implications. Regarding the genotype-phenotype correlation, we observed a high consistency between the resistome profile and VITEK-2 results, as all isolates harboring carbapenemase genes demonstrated phenotypic resistance to carbapenems. However, diagnostically, it complicates phenotypic detection, since co-harboring bla_{NDM} and bla_{OXA-48} family genotypes lead to misidentification of bla_{NDM} when using the Carbapenemase Inactivation Method (mCIM) combined with the EDTA-Carbapenemase Inactivation Method (eCIM) due to a masking effect. This limitation necessitates caution in epidemiological surveillance, particularly in settings relying solely on phenotypic methods without molecular confirmation, as the burden of Metallo-β-lactamases may be significantly underestimated. Therapeutically, this co-carriage threatens the efficacy of last-resort synergistic combinations, specifically ceftazidime-avibactam plus aztreonam. We hypothesize that the specific presence of bla_{OXA-48} family in this genotype might compromise the inhibitory capacity of avibactam, leaving co-produced ESBLs uninhibited to hydrolyze aztreonam, potentially leading to resistance against this final salvage therapy.

In Vietnam, the prevalence of colistin resistance has been reported in *K. pneumoniae* ST15 and ST16, with the primary mechanism involving mutations in the *mgrB* gene that negatively regulate PhoPQ activity [38, 48]. In our study, however, the predominant colistin resistance genomic mechanism was associated with the *pmrB* gene, particularly the R256G mutation. This difference may be attributable to the distribution of various sequence types, including *K. pneumoniae* ST11, ST147, and ST5815. *K. pneumoniae* ST16, the most frequent clone in our study, exhibited these mutations at a much lower frequency, suggesting that its dominance is likely driven by factors other than colistin resistance. In contrast, ST11, ST147, and notably, ST5815 were identified as the primary reservoirs for these mutations. This finding highlights ST5815 as a significant, yet previously under-reported, source of colistin resistance in Southern Vietnam, comparable to ST11 and ST147. The proportion of *mcr*-positive CRE isolates in our study was also low, consistent with previous reports [38].

Our analysis of these CPG-plasmids illustrated a critical co-carrying mechanism. We found that several AMR genes for quinolone (*qnrS1, qnrB6*) and aminoglycoside (*rmtB1, aac(3)-Iid*, *aadA1*) were frequently co-carried on these plasmids carrying CPG. As a result, these isolates are difficult to treat due to an expanded MDR spectrum, rendering therapies such as meropenem combined with an aminoglycoside ineffective. This also suggests that the routine use of common antibiotics (like quinolones) is unintentionally driving the spread of carbapenem resistance [49]. This high-

lights the epidemiological complexity of controlling these MDR reservoirs.

The present findings possess both notable strengths and several limitations. A key strength of this study is that it is the first epidemiological report from Southern Vietnam, sequencing a large number of CRE isolates originating from various infections, and surveying the three most common species among CRE. Nevertheless, these findings must be interpreted within the context of three main limitations. First, our analysis relied on short-read sequencing. While this approach robustly identified co-harbored genes (like *qnrS1*), it could not definitively confirm their physical linkage on the same plasmid. Reconstructing the full architecture of these MDR plasmids would require long-read sequencing. Second, we were unable to distinguish between community-acquired infections and healthcare-associated infections, which limits the analysis of the correlation between isolates in these two settings. Finally, phenotypic AST for colistin was not performed. Therefore, the clinical impact of our genotypic findings (*pmrB* mutations) is based on known mutations, not phenotypic confirmation.

In conclusion, this study provides the first WGS-based molecular epidemiology report on 189 CRE isolates from a major referral center in Southern Vietnam. Clonal expansion of *K. pneumoniae* ST16 and horizontal gene transfer contribute significantly to the dissemination of CPGs. Specific STs possess a unique genomic resistance pathway; specifically, clones carrying bla_{KPC} or bla_{OXA-48} family harbor chromosomal mutations, while clones carrying bla_{NDM} typically lack these mutations. The co-carriage of other critical AMR genes, particularly *qnrS1* and *rmtB1*, within CPG-carrying plasmids significantly expands the MDR phenotype and raises the risk of co-selection through the use of common antibiotics (like ciprofloxacin). These findings underscore the urgent need for national policies and robust infection control strategies to limit the spread of multidrug-resistant pathogens.

Funding

This article was supported by the Cooperative Agreement Number NU2HGH000080, funded by the Centers for Disease Control and Prevention through the Association of Public Health Laboratories. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention, the Department of Health and Human Services, or the Association of Public Health Laboratories. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to express their gratitude to all the members of the study group. We also acknowledge *Dr. Peera Hemarajata* and *Dr. Noah C. Hull* of the Association of Public Health Laboratories for training the sequencing data analysis.

Author contributions

Truong Thien Phu & Tran Trong Tin: contributed equally as first authors to this article in Conceptualization, Methodology, Validation, Formal Analysis, Writing – Original Draft, Writing – review & editing. *Truong Thien Phu*: Supervision, Project Administration. *Tran Trong Tin*: Software, Visualization, Data curation. *Truong Thien Phu, Tran Trong Tin, Le Phuong Mai, Nguyen Van Thanh, Ta Tuan Khanh, Tran Thi Tuyet, Tran Cong Tri, Le Pham My Da, Nguyen Thi Nam Phuong, Nguyen Quang Tin*: Investigation (Material preparation, data collection), Writing – review & editing. All authors read and approved the final manuscript.

Availability of data and materials

All raw reads generated and presented in this study are available at the Sequence Read Archive at NCBI (BioProject: PRJNA1215993).

REFERENCES

[1] Antimicrobial Resistance C. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. 2022; 399(10325): 629-655.

[2] Jean SS, Harnod D, Hsueh PR. Global Threat of Carbapenem-Resistant Gram-Negative Bacteria. *Front Cell Infect Microbiol*. 2022; 12: 823684.

[3] El-Sokkary R, Uysal S, Erdem H, et al. Profiles of multidrug-resistant organisms among patients with bacteremia in intensive care units: an international ID-IRI survey. *Eur J Clin Microbiol Infect Dis*. 2021; 40(11): 2323-2334.

[4] Ayobami O, Brinkwirth S, Eckmanns T, Markwart R. Antibiotic resistance in hospital-acquired ESKAPE-E

infections in low- and lower-middle-income countries: a systematic review and meta-analysis. *Emerg Microbes Infect*. 2022; 11(1): 443-451.
[5] Vu TVD, Do TTN, Rydell U, et al. Antimicrobial susceptibility testing and antibiotic consumption results from 16 hospitals in Viet Nam: The VINARES project 2012-2013. *J Glob Antimicrob Resist*. 2019; 18: 269-278.
[6] Vu TVD, Choisy M, Do TTN, et al. Antimicrobial susceptibility testing results from 13 hospitals in Viet Nam: VINARES 2016-2017. *Antimicrob Resist Infect Control*. 2021; 10(1): 78.
[7] Centers for Disease Control and Prevention. Antimicrobial Resistance Threats in the United States, 2021-2022: CDC; 2024 [updated Jul 16, 2024]. Available at: https://www.cdc.gov/antimicrobial-resistance/data-research/threats/update-2022.html#:~:text=The%20new%20dataA%20show,pre-pandemic%20levels%20in%202022. [accessed 16 Oct 2024].
[8] Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 Guidance on the Treatment of Antimicrobial Resistant Gram-Negative Infections. *Clin Infect Dis.* 2023; 18:ciad428. doi: 10.1093/cid/ciad428.
[9] Grundmann H, Glasner C, Albiger B, et al. Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis*. 2017; 17(2): 153-163.
[10] Paveenkittiporn W, Lyman M, Biedron C, et al. Molecular epidemiology of carbapenem-resistant Enterobacterales in Thailand, 2016-2018. *Antimicrob Resist Infect Control*. 2021; 10(1): 88.
[11] van Duin D, Arias CA, Komarow L, et al. Molecular and clinical epidemiology of carbapenem-resistant Enterobacterales in the USA (CRACKLE-2): a prospective cohort study. *Lancet Infect Dis*. 2020; 20(6): 731-741.
[12] Yin C, Yang W, Lv Y, Zhao P, Wang J. Clonal spread of carbapenemase-producing Enterobacteriaceae in a region, China. *BMC Microbiol*. 2022; 22(1): 81.
[13] The European Committee on Antimicrobial Susceptibility Testing. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Växjö, Sweden: European Committee on Antimicrobial Susceptibility Testing (EUCAST); 2017 July.
[14] Vasquez-Ponce F, Bispo J, Becerra J, et al. Emergence of KPC-113 and KPC-114 variants in ceftazidime-avibactam-resistant Klebsiella pneumoniae belonging to high-risk clones ST11 and ST16 in South America. *Microbiol Spectr*. 2023; 11(5): e0037423.
[15] Maguire M, Serna C, Delgado-Blas JF, et al. Healthcare-related transmission of mobile genetic elements co-carrying bla (NDM) and 16S rRNA methyltransferase genes in multiple Enterobacterales. *Microb Genom*. 2025; 11(8): 001473.
[16] Tavares RDS, Tacao M, Ramalheira E, Ferreira S, Henriques I. Report and Comparative Genomics of an NDM-5-Producing Escherichia coli in a Portuguese Hospital: Complex Class 1 Integrons as Important Players in bla(NDM) Spread. *Microorganisms*. 2022; 10(11): 2243.
[17] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. Wayne, PA, CLSI; 2024.
[18] Libuit KG, Wright SM, Kapsak CJ, et al. Public Health Bioinformatics: Bioinformatics workflows for genomic characterization, submission preparation, and genomic epidemiology of pathogens of public health concern 2023 [updated April 21, 2023]. v2.0.0:[De-novo genome assembly, taxonomic ID, and QC of paired-end bacterial NGS data]. Available at: https://github.com/theiagen/public_health_bioinformatics. [accessed Apr 1 2024]
[19] III RAP. Fastq-scan: GitHub; 2023 [updated October 7, 2022]. v1.0.1:[Output FASTQ summary statistics in JSON format]. Available at: https://github.com/rpetit3/fastq-scan. [accessed Sept 7 2025]
[20] Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014; 30(15): 2114-2120.
[21] Torsten Seemann RE, Goncalves da Silva A, Kiil K. Shovill: GitHub; 2020 [updated March 13, 2020]. Assemble bacterial isolate genomes from Illumina paired-end reads]. Available at: https://github.com/tseemann/shovill. [accessed July 12 2025]
[22] Lumpe J, Gumbleton L, Gorzalski A, et al. GAMBIT (Genomic Approximation Method for Bacterial Identification and Tracking): A methodology to rapidly leverage whole genome sequencing of bacterial isolates for clinical identification. *PLoS One*. 2023; 18(2): e0277575.
[23] Torsten Seemann AS, Goncalves da Silva A. Scan contig files against traditional PubMLST typing schemes: GitHub; 2015 [updated October 28]. v2.23.0:[Scan contig files against PubMLST typing schemes]. Available at: https://github.com/tseemann/mlst. [accessed July 15 2025]
[24] Mikheenko A, Prjibelski A, Saveliev V, Antipov D, Gurevich A. Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics*. 2018; 34(13): i142-i50.
[25] Kislyuk AO, Katz LS, Agrawal S, et al. A computational genomics pipeline for prokaryotic sequencing projects. *Bioinformatics*. 2010; 26(15): 1819-1826.
[26] Page AJ, Cummins CA, Hunt M, et al. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics*. 2015; 31(22): 3691-3693.
[27] Schwengers O, Jelonek L, Dieckmann MA, Beyvers S, Blom J, Goesmann A. Bakta: rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. *Microb Genom*. 2021; 7(11): 000685.
[28] Croucher NJ, Page AJ, Connor TR, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res*. 2015; 43(3): e15.

[29] Hadfield J, Croucher NJ, Goater RJ, Abudahab K, Aanensen DM, Harris SR. Phandango: an interactive viewer for bacterial population genomics. *Bioinformatics*. 2018; 34(2): 292-293.
[30] Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microb Genom*. 2018; 4(8): e000206.
[31] Feldgarden M, Brover V, Gonzalez-Escalona N, et al. AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci Rep*. 2021; 11(1): 12728.
[32] Wang B, Finazzo M, Artsimovitch I. Machine Learning Suggests That Small Size Helps Broaden Plasmid Host Range. *Genes (Basel)*. 2023; 14(11): 2044.
[33] Jain C, Rodriguez RL, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun*. 2018; 9(1): 5114.
[34] Gilchrist CLM, Chooi YH. Clinker & clustermap.js: automatic generation of gene cluster comparison figures. *Bioinformatics*. 2021; 37(16): 2473-2475.
[35] Clinical and Laboratory Standards Institute. Performance Standards For Antimicrobial Disk Susceptibility Tests. 14th ed. Wayne, PA, CLSI; 2024.
[36] Abe R, Akeda Y, Takeuchi D, et al. Clonal dissemination of carbapenem-resistant Klebsiella pneumoniae ST16 co-producing NDM-1 and OXA-232 in Thailand. *JAC Antimicrob Resist*. 2022; 4(4): dlac084.
[37] de Sales RO, Leaden L, Migliorini LB, Severino P. A Comprehensive Genomic Analysis of the Emergent Klebsiella pneumoniae ST16 Lineage: Virulence, Antimicrobial Resistance and a Comparison with the Clinically Relevant ST11 Strain. *Pathogens*. 2022; 11(12): 1394.
[38] Sy BT, Boutin S, Kieu Linh LT, et al. Heterogeneity of colistin resistance mechanism in clonal populations of carbapenem-resistant Klebsiella pneumoniae in Vietnam. *Lancet Reg Health West Pac*. 2024; 51: 101204.
[39] Pham MH, Hoi LT, Beale MA, et al. Evidence of widespread endemic populations of highly multidrug resistant Klebsiella pneumoniae in hospital settings in Hanoi, Vietnam: a prospective cohort study. *Lancet Microbe*. 2023; 4(4): e255-e263.
[40] Andrey DO, Pereira Dantas P, Martins WBS, et al. An Emerging Clone, Klebsiellapneumoniae Carbapenemase 2-Producing K. pneumoniae Sequence Type 16, Associated With High Mortality Rates in a CC258-Endemic Setting. *Clin Infect Dis*. 2020; 71(7): e141-e150.
[41] Arcari G, Carattoli A. Global spread and evolutionary convergence of multidrug-resistant and hypervirulent Klebsiella pneumoniae high-risk clones. *Pathog Glob Health*. 2023; 117(4): 328-341.
[42] Pitout JDD, Peirano G, Kock MM, Strydom KA, Matsumura Y. The Global Ascendency of OXA-48-Type Carbapenemases. *Clin Microbiol Rev*. 2019; 33(1): e00102-00119.
[43] Huang J, Lv C, Li M, et al. Carbapenem-resistant Escherichia coli exhibit diverse spatiotemporal epidemiological characteristics across the globe. *Commun Biol*. 2024; 7(1): 51.
[44] Velasco JM, Valderama MT, Margulieux K, et al. Comparison of Carbapenem-Resistant Microbial Pathogens in Combat and Non-combat Wounds of Military and Civilian Patients Seen at a Tertiary Military Hospital, Philippines (2013-2017). *Mil Med*. 2020; 185(1-2): e197-e202.
[45] Hara Y, Iguchi M, Tetsuka N, et al. Editors' Choice. Multicenter survey for carbapenemase-producing Enterobacterales in central Japan. *Nagoya J Med Sci*. 2022; 84(3): 630-639.
[46] Marimuthu K, Venkatachalam I, Khong WX, et al. Clinical and Molecular Epidemiology of Carbapenem-Resistant Enterobacteriaceae Among Adult Inpatients in Singapore. *Clin Infect Dis*. 2017; 64(suppl_2): S68-S75.
[47] Sisay A, Kumie G, Gashaw Y, Nigatie M, Gebray HM, Reta MA. Prevalence of genes encoding carbapenem-resistance in Klebsiella pneumoniae recovered from clinical samples in Africa: systematic review and meta-analysis. *BMC Infect Dis*. 2025; 25(1): 556.
[48] Bray AS, Smith RD, Hudson AW, et al. MgrB-Dependent Colistin Resistance in Klebsiella pneumoniae Is Associated with an Increase in Host-to-Host Transmission. *mBio*. 2022; 13(2): e0359521.
[49] van Loon K, Voor In 't Holt AF, Vos MC. A Systematic Review and Meta-analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae. *Antimicrob Agents Chemother*. 2018; 62(1): e01730-17.

Supplementary Table S1 - Distribution (n) of Carbapenem-resistant Enterobacterales Sequence Typing across the 12 sampling strata.

	Intensive care unit				*Internal medicine*				*Surgery*			
	Blood	*Sputum/ BAL*	*Urine*	*Others*	*Blood*	*Sputum/ BAL*	*Urine*	*Others*	*Blood*	*Sputum/ BAL*	*Urine*	*Others*
K. pneumoniae												
ST16	8	4	3	8	6	4	8	8	6	4	5	3
ST5815	1	4	-	2	-	3	2	-	2	7	2	2
ST147	2	6	-	1	-	4	-	3	2	-	-	1
ST11	2	1	2	-	-	1	1	3	-	-	1	-
ST656	1	1	-	2	-	-	-	-	-	2	2	-
Other STs	-	1	1	3	2	-	1	-	3	3	2	3
E. coli												
ST410	-	-	-	-	3	2	3	1	1	-	2	1
ST405	1	-	-	-	2	1	1	-	-	-	1	-
Other STs	2	-	-	-	3	2	1	2	-	-	2	2
E. hormaechei												
Other STs	-	-	-	1	-	-	1	-	1	1	1	1
E. cloacae												
Other STs	-	-	-	-	-	-	-	-	1	-	-	-
Total	*17*	*17*	*6*	*17*	*16*	*17*	*18*	*17*	*16*	*17*	*18*	*13*

Abbreviation: - indicates zero occurrences.

Supplementary Table S2 - Complete Colistin Resistance Genotypes (Chromosomal Mutations and Plasmid-mediated *mcr* genes) Identified in Major Sequence Types (STs).

	K. pneumoniae						*E. coli*			*E. hormaechei*	*E. cloacae*
	ST16	*ST5815*	*ST147*	*ST11*	*ST656*	*Other STs*	*ST410*	*ST405*	*Other STs*	*Other STs*	*Other STs*
*pmrB*_R256G	-	25	18	7	-	3	-	-	-	-	-
*pmrB*_Y358N	-	-	-	-	-	-	13	-	1	-	-
*pmrB*_E123D	-	-	-	-	-	-	-	-	3	-	-
mcr-8.2, *pmrB*_R256G	-	-	-	2	-	-	-	-	-	-	-
*pmrB*_T157P	2	-	-	-	-	-	-	-	-	-	-
*crrB*_G183V	1	-	-	-	-	-	-	-	-	-	-
mcr-1.1	-	-	-	-	-	-	-	1	-	-	-
mcr-8, *pmrB*_R256G	-	-	-	1	-	-	-	-	-	-	-
*mgrB*_Q30STOP, *pmrB*_R256G	-	-	-	-	-	1	-	-	-	-	-
*pmrA*_G53S, *pmrB*_R256G	-	-	-	1	-	-	-	-	-	-	-
*pmrB*_R256G, *pmrB*_T157P	-	-	1	-	-	-	-	-	-	-	-
*pmrB*_T140P	1	-	-	-	-	-	-	-	-	-	-
Not detected	63	-	-	-	8	15	-	5	10	6	1
Total	*67*	*25*	*19*	*11*	*8*	*19*	*13*	*6*	*14*	*6*	*1*

Abbreviation: - indicates zero occurrences.