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Original Article

The clonality and dissemination of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Malaysia: a retrospective analysis from 2022 to 2023

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ABSTRACT

Objectives: Cases of nosocomial carbapenem-resistant *Klebsiella pneumoniae* (CRKP) have been increasing steadily since the 1990s. In this study, we sought to assess CRKP clonal diversity and patterns of dissemination at Hospital Canselor Tuanku Muhriz (HCTM), a tertiary university hospital located in Kuala Lumpur, Malaysia.
Methods: From January 2022 to December 2023, all CRKP isolates from HCTM were included in the investigation. Associated patient demographic data and clinical histories were retrieved from hospital records. Antibiotic susceptibility data for the collected isolates were obtained from the HCTM diagnostic laboratory. Molecular typing was performed using enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) to identify genetic clusters. Based on ERIC-PCR clustering results and epidemiological investigations, selected CRKP isolates were subjected to whole-genome sequencing (WGS) to validate clonal relatedness, identify antimicrobial resistance genes, and establish phylogenomic relationships.
Results: During the study period, a total of 147 CRKP isolates were recovered from various wards across HCTM. All isolates exhibited resistance to imipenem, meropenem, or ertapenem, with 124 isolates confirmed as carbapenemase producers. ERIC-PCR identified 24 genetic clusters disseminated across 23 wards within HCTM. Twelve CRKP isolates were selected for WGS based on integrated epidemiological investigation and ERIC-PCR genotyping; sequence typing revealed ST17 as the dominant circulating CRKP lineage in the intensive care unit, ward 6E, and possibly across the hospital. The carbapenemase gene *bla_NDM* was detected in all WGS-analyzed CRKP isolates.

Conclusion: Integrating epidemiological investigation, phenotypic testing, molecular typing, and WGS is essential to understand resistance dynamics, map transmission, and guide hospital infection prevention strategies.

Keywords: Carbapenem; Carbapenem-resistant Enterobacteriaceae; *Klebsiella pneumoniae*; Molecular typing; Polymerase chain reaction; Whole genome sequencing

Introduction

In both global and Malaysian contexts, *Klebsiella pneumoniae* is the predominant species among carbapenem-resistant Enterobacterales (CRE). Carbapenem-resistant *K. pneumoniae* (CRKP) represents a major global public health challenge, particularly in healthcare-associated settings [1]. Surveillance data from Malaysia, as reported by the country's National Health Institute, underscore the growing threat posed by CRKP [2,3]. The resistance rate of *K. pneumoniae* to meropenem increased from 2.3% in 2018 to 5.0% in 2022, indicating a concerning upward trend. This increase is largely driven by the dissemination of carbapenemase-producing genes such as *bla_NDM* and *bla_OXA-48*, with *bla_NDM* being the most frequently identified among Malaysian CRE isolates [3].

CRKP poses a substantial clinical threat, driven primarily by the production of carbapenemase enzymes that render most β-lactam antibiotics ineffective and severely limit available treatment options. To elucidate the transmission dynamics of a prolonged CRKP outbreak at Hospital Canselor Tuanku Muhriz (HCTM), Universiti Kebangsaan Malaysia, a tertiary university hospital located in Cheras, Kuala Lumpur, we conducted a genomic epidemiology investigation integrating epidemiological data, enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping, and whole-genome sequencing (WGS) of circulating CRKP strains isolated from the hospital.

HIGHLIGHTS

- A total of 147 carbapenem-resistant *Klebsiella pneumoniae* isolates were analyzed from a tertiary university hospital between 2022 and 2023.
- Enterobacterial repetitive intergenic consensus polymerase chain reaction enterobacterial repetitive intergenic consensus polymerase chain reaction suggested clonal relatedness among isolates, which was confirmed using whole-genome sequencing (WGS).
- *bla_NDM* was identified in all WGS isolates, underscoring the role of mobile genetic elements in resistance spread.
- The findings highlight the need for a combined approach to infection control in critical care settings.

Materials and Methods

Study Setting and Data Collection

This retrospective study was conducted at HCTM. The hospital has an infection prevention and control unit (IPCU) responsible for routine surveillance, infection prevention activities, staff training, and outbreak investigations. Routine IPCU surveillance data collected between January 1, 2022, and December 31, 2023, were reviewed. For each CRKP infection, information on patient demographics, ward or unit of admission, clinical diagnosis, and specimen type was retrieved.

Bacterial Identification and Antimicrobial Susceptibility Testing

CRKP identification was performed by HCTM's Department of Diagnostic Laboratory Services (JPMD) using conventional biochemical tests and API 20E (bioMérieux) prior to the implementation of the automated VITEK 2 Compact system (bioMérieux) in July 2023, which utilized AST-N374 cards for Gram-negative organisms. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disc diffusion method on Mueller-Hinton agar for selected agents from the penicillin, cephalosporin, carbapenem, aminoglycoside, and fluoroquinolone classes and was interpreted according to Clinical and Laboratory Standards Institute (CLSI) M100 guidelines [4]. Multidrug resistance was defined as non-susceptibility to at least 1 agent in 3 or more antimicrobial classes. For isolates with reduced susceptibility to carbapenems, minimum inhibitory concentrations for imipenem, meropenem, and ertapenem were determined using Etest gradient strips (bioMérieux).

Carbapenemase Detection and Classification

Carbapenemase production was assessed using the modified carbapenem inactivation method (mCIM) and, where applicable, the ethylenediaminetetraacetic acid-modified

carbapenem inactivation method (eCIM), in accordance with CLSI guidelines [4]. eCIM testing was performed for isolates that tested positive by mCIM.

ERIC-PCR and Cluster Identification

ERIC-PCR genotyping was performed for all 147 CRKP isolates collected during the study period using ERIC primers (forward: 5′-ATG TAA GCT CCT GGG GAT TCAC-3′ and reverse: 5′-AAG TAA GTG ACT GGG GTG AGC G-3′) [5]. The PCR protocol consisted of an initial denaturation at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 48 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. PCR products were resolved on 1.5% agarose gels at 70 V for 1 hour and visualized under ultraviolet light. DNA fingerprint patterns generated by ERIC-PCR were analyzed using GelCompar II software to construct dendrograms based on unweighted pair group method with arithmetic mean clustering and dice similarity coefficients. Clinical and epidemiological data were cross-referenced to identify genetic clusters.

WGS, Genome Assembly, and Annotation

Selected strains representing ERIC-PCR clusters were subjected to further analysis using WGS. Genomic DNA extraction and purification were performed by bead homogenization using the ZymoBIOMICS DNA Extraction Kit (Zymo Research), according to the manufacturer's instructions. For library preparation, 100 ng of genomic DNA was fragmented to approximately 350 bp using a Bioruptor and processed with the NEB Ultra II Library Preparation Kit (New England BioLabs) following the manufacturer's protocol. Sequencing was conducted on an Illumina NovaSeq 6000 platform using a 2×150 bp paired-end configuration, generating approximately 1 Gb of sequencing data per isolate. Genome assembly was performed using SPAdes v4 [6]. Genome annotation was carried out using Bacterial Annotation Toolkit (BAKTA) v1.9.3 with its standard database [7], in which protein-coding genes were predicted using Prodigal v2.6.0 [8] and annotated against multiple databases, including National Center for Biotechnology Information (NCBI) RefSeq, UniProt, Pfam, Rfam, KEGG, and Gene Ontology. Protein sequences were additionally submitted to the eggNOG-mapper and KofamKoala web servers [9,10].

High-Resolution Taxonomic Classification, Single-Nucleotide Polymorphism-Tree Construction, Multi-Locus Sequence Typing, and Antimicrobial Resistance Gene Prediction

For taxonomic classification, pairwise average nucleotide identity was calculated between genome assemblies and reference genomes in the GTDB r220 database using FastANI v1.33 [11,12]. Genome assemblies were further used to construct a single-nucleotide polymorphism (SNP)-based phylogenetic tree using maast v1.0.8 with default parameters [13], in which SNPs were identified through pairwise comparisons to generate concatenated allele alignments. These nucleotide alignments were used as input for maximum-likelihood phylogenetic tree construction with FastTree 2 v2.1.11 [14]. CRKP sequence types (STs) were assigned according to the PubMLST database using the standard 7-locus multi-locus sequence typing (MLST) scheme (gapA, infB, mdh, pgi, phoE, rpoB, and tonB), as implemented in the mlst tool [15]. To identify antimicrobial resistance (AMR) genes, assembled genomes were searched against the NCBI AMRFinder, ResFinder, and CARD databases using Abricate v1.0.0 [16–18].

Integrated Epidemiological, Genotyping, and Genomic Analyses for CRKP Transmission Identification

Clinical and epidemiological data for WGS-selected isolates, including patient medical history, diagnosis, specimen type, ward or unit, and collection date, were reviewed in conjunction with ERIC-PCR genotyping and genomic findings (MLST, SNP, and AMR analyses) to infer potential transmission trajectories. Cases associated with strains exhibiting similar ERIC-PCR genotypes, STs, SNP profiles, and AMR gene content that also shared temporal overlap (ward admission periods) and spatial proximity (patient ward movement) were considered indicative of transmission events. In contrast, genetic or genomic dissimilarity among isolates with epidemiological overlap suggested independent introductions rather than direct transmission. This integrated analytical framework enabled higher-resolution mapping of CRKP transmission and provided critical insights into both clonal persistence and the coexistence of genetically diverse strains within HCTM.

Ethics Approval

This study was approved by the Medical Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2023-869).

Results

Most CRKP Isolates Were Multidrug-Resistant and Carbapenemase Producers

Thirty-eight and 109 cases of CRKP infections were recorded at HCTM in 2022 and 2023, respectively, resulting in the

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isolation of 147 CRKP isolates (Table S1). The median age of patients from whom CRKP was isolated was 51.5 years (range, 14–89 years), and most patients were admitted to the general intensive care unit (GICU) (n=46). Most CRKP isolates were recovered from blood specimens (n=50) (Table S1).

All study isolates were multidrug resistant, with susceptibility largely restricted to gentamicin and amikacin (81.5% and 82.1% susceptibility to gentamicin in 2022 and 2023, respectively; 78.4% and 92.2% susceptibility to amikacin in 2022 and 2023, respectively). A total of 124 isolates (84.4%) were carbapenemase producers. Ten isolates (6.8%) were mCIM-negative and classified as non-carbapenemase-producing CRKP. Twelve isolates (8.2%) were not tested because of the unavailability of mCIM reagents at the time of analysis. Among the carbapenemase-producing isolates, 87 (70.2%) were identified as metallo-β-lactamase producers (eCIM positive), whereas 5 (4.0%) were classified as serine carbapenemase producers (eCIM negative). eCIM testing was not performed for 32 isolates (25.8%), as testing was conducted for epidemiological surveillance rather than therapeutic guidance.

Diverse CRKP Genotypes Identified by ERIC-PCR with Inter-Ward Distribution

ERIC-PCR dendrogram analysis revealed 24 clusters at a 50% similarity threshold (Figures S1, S2). Strains within clusters 10, 11, 22, and 24 demonstrated closer similarity (>60%). No cluster was confined to a single ward, indicating the dissemination of multiple CRKP genotypes across hospital wards. Several isolates were further analyzed based on genotype similarity and epidemiological investigation (Figure 1).

Improved Detection of CRKP Transmission Using Combined Epidemiological, Clinical, ERIC-PCR, and Genomic Approaches

Twelve CRKP isolates were selected for WGS based on ERIC-PCR-defined clustering and epidemiological linkage. Genome assembly statistics and genome completeness information are provided in Table S2 and Figure S3.

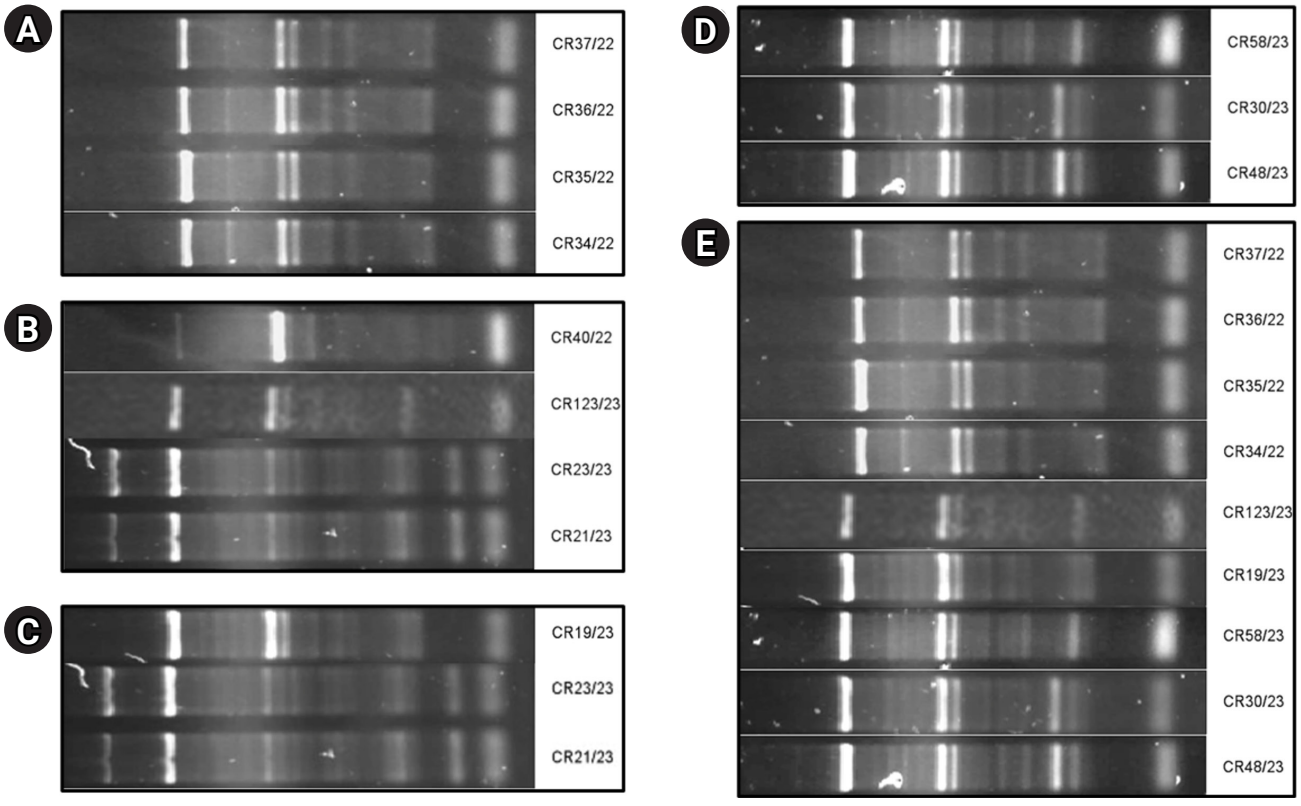

Figure 1. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) dendrograms of selected carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates investigated in this study. (A) A cluster comprising isolates CR34/22, CR35/22, CR36/22, and CR37/22. (B) Selected isolates from the general intensive care unit. (C) CR19/23 exhibited a different ERIC-PCR profile from CR21/23 and CR23/23, despite close contact between the patients associated with these isolates. (D) A cluster isolated from ward 6E, comprising CR58/23, CR30/23, and CR48/23. (E) All ST17 isolates were found to share the same ERIC-PCR profile. ST, sequence type.

First, 4 isolates from ERIC-PCR cluster 22 (CR34/22, CR35/22, CR36/22, and CR37/22), collected from a single intensive care unit (ICU) patient in 2022 (Table 1; Figure 1A), were investigated. In addition to sharing similar ERIC-PCR genotypes, WGS revealed that these 4 isolates shared identical STs and highly similar AMR gene profiles (Figure 2). This finding was consistent with persistent CRKP infection or colonization in a single patient during a prolonged ICU stay from late August to mid-September 2022, with multiple clinical specimens yielding isolates that were highly similar at the whole-genome level (Figure 3).

Next, several ERIC-PCR genotypes (that of CR40/22, CR21/23, CR23/23, CR123/23) (Table 1; Figure 1B) corresponding to CRKP isolates recovered from the GICU over the study period (2022–2023) were analyzed. The ERIC-PCR profile of CR40/22 was initially unclear; however, WGS identified this isolate as ST4276 with a distinct AMR gene profile compared with other sequenced CRKP isolates (Figure 2). In contrast, ERIC-

Table 1. Epidemiological, clinical, ERIC-PCR genotyping, and genomic data for WGS isolates

Sample ID	Patient ID	Close contact (if any)	Specimen type	Collection date	Ward of CRKP isolation	Patient ward movement prior to CRKP isolation	Patient medical history	ERIC-PCR cluster	ST
CR34/22	A		Blood	August 21, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR35/22	A		Urine	August 24, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR36/22	A		Urine	August 27, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR37/22	A		Urine	September 7, 2022	GICU	12/7 (5D) 19/7 (GICU) 26/7 (5H) 28/7 (COVID ICU)	Retropharyngeal abscess with COVID-19 pneumonia	22	17
CR40/22	B		Blood	September 23, 2022	GICU	22/7 (GICU)	Septic shock secondary to HAP	9	4,276
CR19/23	C	Close contact in ICU	Tracheal aspirate	April 11, 2023	GICU	30/3 (4H) 2/4 (GICU)	Severe pancreatitis with multiorgan failure	11	17
CR21/23	D		Rectal swab (routine screening)	April 17, 2023	GICU	13/4 (GICU)	Severe pancreatitis with multiorgan failure	10	11
CR23/23	D		Tracheal aspirate	April 20, 2023	GICU	13/4 (GICU)	Severe pancreatitis with multiorgan failure	10	11
CR123/23	E		Urine	October 9, 2023	GICU	13/9 (GICU)	Extensive left cerebellar infarct	10	17
CR30/23	F	Close contact with D in ICU	Tracheal aspirate	May 5, 2023	Ward 6E	14/4 (7C) 17/4 (COVID ICU) 20/4 (GICU) 29/4 (6E)	Post COVID-19 category 5	10	17
CR48/23	G		Rectal swab	June 7, 2023	Ward 6E	17/4 (5C) 22/4 (6E) 19/5 (RICU) 23/5 (6E)	Sepsis secondary to HAP	10	17
CR58/23	H		Blood	June 16, 2023	Ward 6E	7/6 (4F)	Proximal myositis	10	17

ERIC-PCR, enterobacterial repetitive intergenic consensus polymerase chain reaction; WGS, whole-genome sequencing; CRKP, carbapenem-resistant *Klebsiella pneumoniae*; ST, sequence type; GICU, general intensive care unit; ICU, intensive care unit; COVID-19, coronavirus disease 2019; HAP, hospital-acquired pneumonia; RICU, respiratory intensive care unit.

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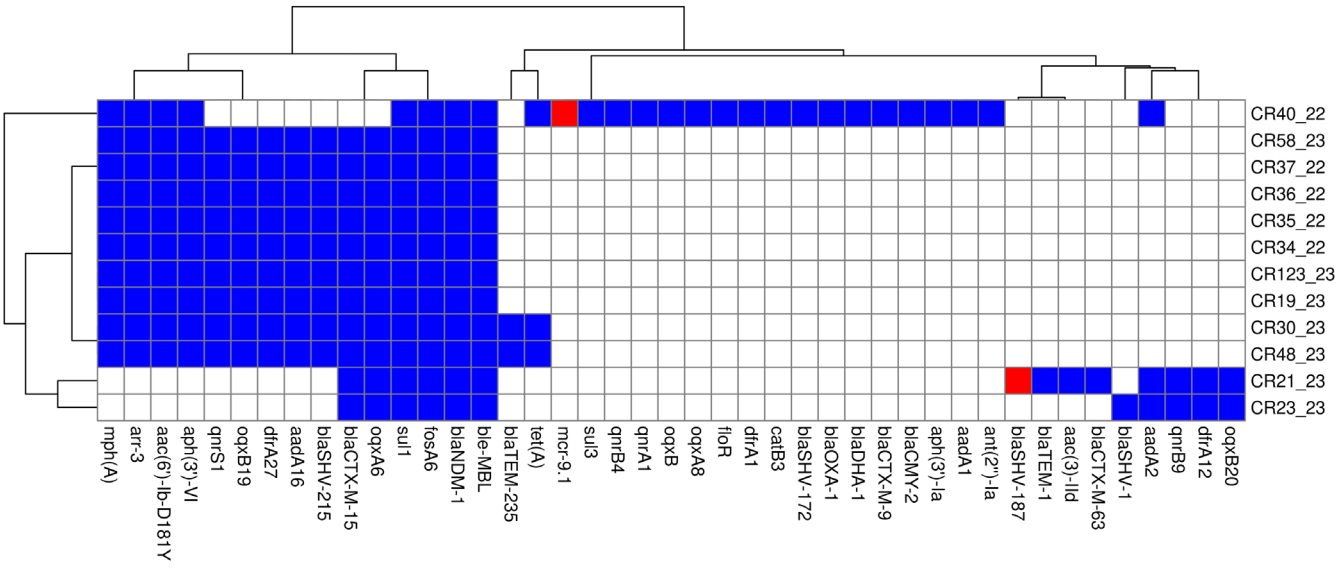

Figure 2. Heatmap of antimicrobial resistance (AMR) gene profiles of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) isolates subjected to whole-genome sequencing. Each cell represents the presence or absence of a specific AMR gene in each genome. Dark blue indicates genes detected with 100% sequence identity, red indicates genes detected with <100% sequence identity (partial variant matches), and white indicates absence of the gene. All sequenced isolates carried the *bla_NDM* gene, confirming their classification as CRKP. While *bla_NDM* was universally present, other resistance determinants such as *aac(6')-Ib* and *oqxA* were variably detected, reinforcing that the CRKP isolates were not derived from a single clone.

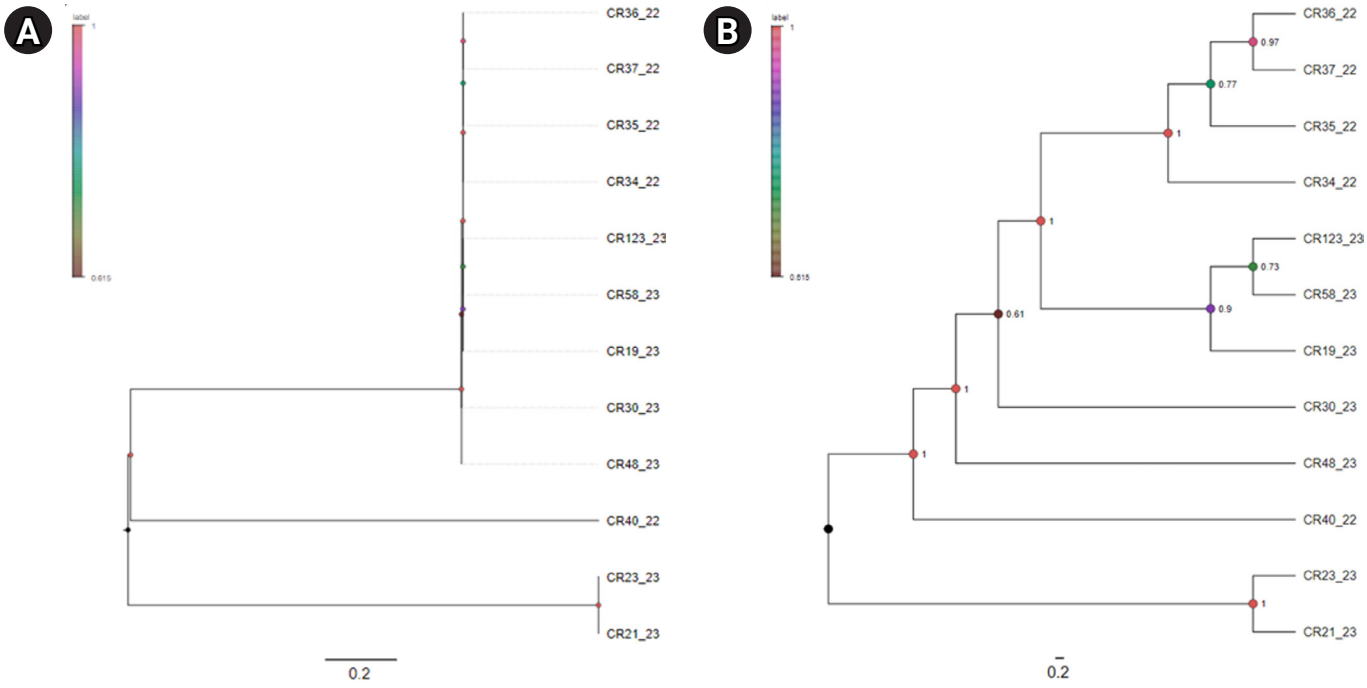

Figure 3. Maximum-likelihood phylogenomic analysis demonstrated that carbapenem-resistant *Klebsiella pneumoniae* isolates subjected to whole-genome sequencing did not belong to a single clone. (A) Although the majority were classified as ST17, genetically distinct ST4276 and ST11 lineages were also identified. (B) Isolates recovered in 2022 appeared to have diverged more recently from a ST17 ancestor than those recovered in 2023. These findings underscore the complexity of transmission dynamics in high-risk hospital settings, where clonal evolution and expansion may coexist with sporadic introductions, complicating transmission tracking efforts. ST, sequence type.

PCR genotypes were indistinguishable for isolates CR21/23 and CR23/23 (Figure 1B). These isolates were recovered from the same patient (patient D) in April 2023 and were later confirmed by WGS to belong to ST11 and to cluster on the same branch of the SNP-based phylogenomic tree (Figure 3A), differing by only a small number of SNPs (Figure 3B). Intriguingly, CR23/23, which was isolated 3 days later than CR21/3, did not carry *blaCTX-M-63*, *blaTEM-1*, *aac(3)-IId*, or *bla-SHV-187*, but instead harbored *blaSHV-1* (Figure 2). Based on epidemiological investigation, the IPCU included the ERIC-PCR genotype of CR19/23, an isolate from patient C (a close contact of patient D in the ICU), for further ERIC-PCR and WGS analysis (Figure 1C). CR19/23 was found to exhibit a different ERIC-PCR genotype from CR21/23 and CR23/23 and was confirmed by WGS to be phylogenomically distant from these isolates (Figure 3). Further epidemiological investigation revealed that CR21/23, the first isolate recovered from patient D, originated from routine rectal swab screening and belonged to ERIC-PCR cluster 10 (ST11). This isolate likely originated from the community, was not hospital-associated, and did not appear to spread beyond its initial host (patient D). In contrast, CR19/23 and CR123/23 were identified as ST17 and shared a common ancestor with CRKP isolates recovered in 2022 (Figure 3B). Overall, ST17 CRKP isolates recovered between August 2022 and October 2023 belonged to closely related ERIC-PCR clusters (clusters 10, 11, and 22) (Table 1; Figure S2) and were predominantly isolated from the ICU, suggesting a prolonged outbreak of ST17 CRKP in this setting.

CR30/23, CR48/23, and CR58/23 from ERIC-PCR cluster 10 (Table 1; Figure 1D) were isolated from patients F, G, and H, respectively, during an outbreak in ward 6E in May and June 2023. Epidemiological contact tracing indicated that patient F (CR30/23) had close contact with patient D (CR21/23 and CR23/23) during their ICU stay, initially raising suspicion of CRKP transmission from the ICU to ward 6E. However, WGS demonstrated that CR30/23, CR48/23, and CR58/23 were ST17 and shared a more recent phylogenomic ancestor with each other than with CR21/23 and CR23/23 (Figure 3), an indication that the hypothesis of transmission from patient D to patient F was incorrect. Indeed, all ST17 CRKP isolates subjected to WGS exhibited similar ERIC-PCR genotypes and were assigned to clusters 10, 11, or 22, which shared >60% similarity (Figure 1E), highlighting the utility of ERIC-PCR as a screening tool for WGS strain selection in resource-limited hospital settings. Chronologically, patients F (CR30/23) and G (CR48/23) may have acquired ST17 CRKP in the ICU and subsequently transmitted the organism to patient H (CR58/23) during their stay in ward 6E. However, CR30/23 and CR48/23 were the only WGS isolates that carried *tet(A)* and *blaTEM-235* (Figure 2), suggesting independent acquisition of these AMR determinants. In addition, CR58/23 appeared to have diverged more recently from the ST17 ancestor than CR30/23 and CR48/23 (Figure 3B). Sequencing additional isolates across the study period would allow a more comprehensive understanding of the evolutionary and transmission dynamics of ST17 CRKP.

Discussion

This study demonstrates the complementary yet critical roles of ERIC-PCR and WGS in the investigation of a CRKP outbreak in our university hospital. ERIC-PCR provided a rapid and cost-effective means of clustering isolates, making it a practical frontline screening tool in resource-limited settings [19–21]. However, poor gel image resolution, as observed for isolate CR40/22, may result from uneven PCR amplification of specific genomic regions and can complicate genotype assignment. In such situations, WGS offers substantially higher resolution and reveals genetic differences that ERIC-PCR cannot capture, consistent with previous reports highlighting the superiority of WGS for accurately delineating transmission events [22,23]. In our study, there was initial suspicion of CRKP transmission between patients C (CR19/23, ST17) and D (CR21/23 and CR23/23, both ST11), and subsequently between patients D (CR21/23 and CR23/23) and F (CR30/23) based on epidemiological investigation; notably, WGS demonstrated that these transmission events had not occurred. This finding further strengthened the confidence of our IPCU in applying WGS for future close-contact assessments and outbreak investigations.

A notable finding of this study was the persistence of CRKP ST17 across multiple time points during the study period, with possible horizontal acquisition of AMR mobile genetic elements in some isolates (CR30/23 and CR48/23). This persistence suggests that ST17 did not represent a short-lived outbreak but rather an endemic lineage entrenched within the ICU environment, with possible subsequent spread to ward 6E. Its prolonged survival points toward maintenance through environmental reservoirs and gaps in infection control measures [24]. In contrast, lineages such as ST4276 and ST11 were detected only once, suggesting sporadic introductions rather than sustained persistence. The contrast between these 2 patterns illustrates the dual challenge faced by infection prevention teams: controlling both endemic high-risk clones and newly introduced strains.

The ICU setting plays a central role in facilitating both the persistence and spread of CRKP. Patients frequently undergo invasive procedures, receive broad-spectrum antibiotics, and rely on multiple medical devices, all of

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which increase susceptibility to colonization and infection. The coexistence of clonally related isolates such as ST17 with strains that appear to have independently acquired AMR determinants (e.g., CR30/23 and CR48/23 harboring *tet(A)* and *bla_TEM-235*) reflects the complex interplay between clonal expansion and repeated acquisition events, a pattern also observed in other high-risk settings [25,26]. These findings highlight the limitations of traditional epidemiological contact tracing. While epidemiological links provide valuable insights, they cannot fully capture the complexity of transmission dynamics, particularly in the presence of independent introductions. By integrating molecular tools such as ERIC-PCR and WGS into outbreak investigations, transmission pathways can be more accurately resolved, allowing infection prevention and control (IPC) teams to distinguish between clonal dissemination and multiple acquisition events [27–29].

The dissemination of ST17 from the ICU to ward 6E underscores the importance of hospital-level, rather than ward-specific, IPC strategies. Shared healthcare personnel, contaminated equipment, and patient transfers create opportunities for inter-ward spread, consistent with previous reports describing cross-unit dissemination of multidrug-resistant organisms [30,31]. Measures such as strict adherence to hand hygiene, environmental decontamination, and the use of dedicated medical devices for high-risk wards should therefore be prioritized [32–36]. In addition, inappropriate or excessive use of carbapenems may select for diverse resistant lineages, indicating that antimicrobial stewardship programs tailored to local resistance patterns are essential [37,38].

An important limitation in low-resource settings is the inability to perform WGS on all isolates because of financial and logistical constraints [39]. In such contexts, ERIC-PCR serves as a valuable complementary method for rapidly and cost-effectively screening large numbers of isolates, thereby prioritizing representative samples for sequencing [40]. This tiered approach enables a balance between resolution and feasibility, ensuring that outbreak investigations can still generate meaningful insights even when resources are constrained [41–43].

Overall, the persistence of ST17 in the ICU in our study illustrates how high-risk clones can establish long-term reservoirs in critical care environments, where they function as both amplifiers and sources of hospital-wide dissemination and potentially environmental contamination [30,31]. Sustained control of CRKP will require continuous genomic surveillance and bacterial characterization, ideally using WGS [44,45], integration of ERIC-PCR and WGS into routine workflows, and alignment of IPC and antimicrobial stewardship interventions [46]. Future studies should focus on real-time sequencing and prospective epidemiological investigations to better elucidate the contributions of asymptomatic carriers and environmental reservoirs to the persistence of high-risk clones such as ST17.

Conclusion

This study demonstrates the value of combining ERIC-PCR with WGS to refine investigations of CRKP outbreaks in our hospital. While ERIC-PCR offers a rapid and inexpensive means of clustering isolates, WGS provides high-resolution typing that distinguishes clonal spread from independent acquisition events. Nonetheless, ERIC-PCR remains a valuable frontline tool in low-resource settings for prioritizing isolates for WGS. Adoption of such a tiered approach allows hospitals to balance feasibility with analytical precision, enabling earlier detection, more accurate outbreak mapping, and targeted interventions. Integrating molecular epidemiology with robust infection control and antimicrobial stewardship efforts is essential to contain the threat of CRKP and mitigate its long-term impact on patient safety.

Supplementary Material

Table S1. Patient demographics and information on the 147 carbapenem-resistant *Klebsiella pneumoniae* isolates included in this study; **Table S2.** Genome assembly statistics for 12 carbapenem-resistant *Klebsiella pneumoniae* isolates subjected to whole-genome sequencing; **Figure S1.** Enterobacterial repetitive intergenic consensus polymerase chain reaction genotyping for all 147 carbapenem-resistant *Klebsiella pneumoniae* isolates of the study; **Figure S2.** Clustering of enterobacterial repetitive intergenic consensus polymerase chain reaction genotypes for study isolates. A total of 24 clusters were identified as having >50% similarity. Strains in clusters 10, 11, 22, and 24 appeared to have closer similarity (>60%). No cluster was distinct to a single ward, signifying transmission of various carbapenem-resistant *Klebsiella pneumoniae* genotypes across wards of the hospital; **Figure S3.** Benchmarking Universal Single-Copy Orthologs (BUSCO) completeness assessment of the assembled genomes using BUSCO v6 with the Enterobacterales_odb12 lineage dataset (n=874 orthologs). Bars indicate the proportion of single-copy complete (S, light blue), duplicated complete (D, dark blue), fragmented (F, yellow), and missing (M, red) BUSCOs. All genomes showed very high completeness (>98% complete BUSCOs), with only a small fraction of fragmented or missing orthologs, reflecting the overall high quality and completeness of the genome assemblies. Supplementary data are available at

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Notes

Ethics Approval

This study was approved by the Medical Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2023-869) and conducted at Hospital Canselor Tuanku Muhriz in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived due to the retrospective nature of the study.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

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Availability of Data

The whole-genome sequencing (WGS) project for *Klebsiella pneumoniae* isolates analyzed in this study has been deposited in the DDBJ/ENA/GenBank under the BioProject accession PRJNA1333618. Individual genome sequences are available under accession numbers JBROFO000000000–JBROFZ000000000 (BioSamples SAMN51830973–SAMN51830984). The version described in this paper is version JBROFO010000000–JBROFZ010000000.

Raw sequencing data and genome assemblies generated from this study have been submitted under the BioProject PRJNA1333618.

Authors' Contributions

Conceptualization: UAZ, PP, SAS, HN; Data curation: UAZ, PP, XKC, NK, CLL, SAS, SS, NAAF, MFNY, HMG, HN, SRY; Formal analysis: UAZ, PP, HN, SAS, HMG; Funding acquisition: UAZ, PP, HN; Investigation: SAS, SS, UAZ, HN; Methodology: UAZ, HN; Project administration: all authors; Resources: all authors; Software: UAZ, HMG, SAS, HN; Supervision: PP, SAS, HN; Validation: PP, SAS, HMG, HN; Visualization: UAZ, HN; Writing–original draft: UAZ, HN; Writing–review & editing: all authors. All authors read and approved the final manuscript.

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