Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

GENOMIC EPIDEMIOLOGY OF CARBAPENEM-RESISTANT *KLEBSIELLA PNEUMONIAE*: INTEGRATING RESISTANCE GENES, VIRULENCE DETERMINANTS, AND CLINICAL OUTCOMES

Muskan Naseer [1], Ayesha Khaliq [2], Malaika Jameel Butt*[3]

[1]*Department of Biological Sciences, Superior University Lahore*
[2, *3]*Institute of Molecular Biology and Biotechnology, The University of Lahore*

[1]muskanmalik35401@gmail.com, [2]ayeshakhaliq306@gmail.com, [*3]malaikajameelbutt@gmail.com

DOI: https://doi.org/10.5281/zenodo.23760535

Keywords
carbapenem-resistant Klebsiella pneumoniae; genomic epidemiology; carbapenemase; ceftazidime–avibactam; convergent hypervirulence; mortality

Article History
Received: 20 January 2026
Accepted: 05 March 2026
Published: 19 March 2026

Copyright @Author
Corresponding Author: *
Malaika Jameel Butt

Abstract

Background: *Carbapenem-resistant Klebsiella pneumoniae (CRKP) is a critical-priority pathogen, yet how carbapenemase mechanism, co-resistance to last-line agents, and the emergence of convergent resistance–virulence lineages jointly shape clinical outcomes remains incompletely resolved. We integrated genomic determinants with phenotypic resistance and clinical data to identify independent predictors of mortality.*

Methods: *We analysed 200 non-duplicate CRKP isolates from invasive and respiratory infections across three hospitals (2021–2024). Each isolate was characterised for sequence type (ST), clonal group (CG), capsular (KL) and O-antigen loci, carbapenemase class and allele, acquired resistance and porin determinants, and a defined virulence-locus panel (rmpA/rmpA2, aerobactin, salmochelin, yersiniabactin, colibactin, peg-344). A convergent genotype was defined as co-carriage of a carbapenemase with a hypervirulence-associated siderophore or regulator locus. Minimum inhibitory concentrations (MICs) were determined for seven agents. Associations with 30-day mortality were assessed by logistic and Cox proportional-hazards regression.*

Results: *Thirty-day mortality was 32.5% (65/200). KPC (33.5%) and OXA-48-like (30.5%) predominated, followed by metallo-β-lactamases (MBLs: NDM, VIM, IMP; 27.5%). All isolates were ceftazidime-resistant and 85.0% meropenem-resistant. Ceftazidime–avibactam resistance (30.5% overall) was almost entirely explained by MBL production (100% of MBL producers vs 4.1% of non-MBL isolates; p<0.001). Convergent resistance–virulence genotypes were present in 22.0% of isolates and carried higher 30-day mortality than non-convergent isolates (45.5% vs 28.8%; log-rank p=0.020). In multivariable analysis, independent predictors of 30-day mortality were patient age (adjusted OR [aOR] 1.03 per year, 95% CI 1.00–1.05), Pitt bacteraemia score (aOR 1.29, 1.07–1.56), ceftazidime–avibactam resistance (aOR 2.11, 1.06–4.20), and convergent genotype (aOR 2.28, 1.08–4.85). The convergent genotype remained independently associated with mortality in Cox analysis (HR 2.03, 1.17–3.54).*

Conclusions: *In this integrated genomic-epidemiological cohort, CRKP mortality was driven less by carbapenem resistance per se than by the loss of ceftazidime*

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

avibactam activity in MBL producers and by the convergence of carbapenem resistance with hypervirulence determinants. Genomic surveillance that reports carbapenemase class and virulence convergence not carbapenem resistance alone offers actionable prognostic and stewardship value.

1. Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) sits at the top of the World Health Organization's priority-pathogen list and is among the leading causes of attributable deaths from antimicrobial resistance worldwide (1). Invasive CRKP infection carries crude mortality of 30–50% in most case series, and therapeutic options are constrained to a small number of last-line agents whose activity is itself eroding (2). Understanding *why* some CRKP infections prove lethal while others are survivable is therefore a question of direct clinical and public-health consequence (3). The genomic architecture underlying CRKP is heterogeneous in three respects that are usually studied in isolation. First, carbapenem resistance is mediated by mechanistically distinct enzymes serine carbapenemases (KPC), OXA-48-like oxacillinases, and metallo-β-lactamases (MBLs: NDM, VIM, IMP) that differ critically in their susceptibility to β-lactamase inhibitors (4). Ceftazidime–avibactam, a mainstay of modern CRKP therapy, retains activity against KPC and OXA-48-like producers but is intrinsically inactive against MBLs, so carbapenemase *class*, not merely its presence, governs whether a frontline regimen will work (5). Second, *K. pneumoniae* lineages carry a spectrum of virulence determinants the regulators *rmpA/rmpA2* and the siderophore systems aerobactin, salmochelin and yersiniabactin historically partitioned between "classical" multidrug-resistant and "hypervirulent" community strains (6). Third, this partition is breaking down: convergent lineages that combine carbapenemases with hypervirulence loci have been reported globally and are feared to unite transmissibility, resistance and invasiveness in a single organism (7). Most published cohorts analyse these axes separately resistance genotype, virulence genotype, or clinical outcome and the few integrative studies are frequently underpowered for the multivariable modelling needed to separate pathogen genomics from host and treatment factors (8). As a result, it remains unclear whether the convergence of resistance and virulence carries measurable prognostic weight once host severity and appropriate therapy are accounted (9) for, and whether the loss of ceftazidime–avibactam activity in MBL producers translates into worse survival at the bedside. We addressed these gaps with an integrated genomic-epidemiological analysis of 200 CRKP isolates, linking carbapenemase class and allele, a curated virulence-locus panel, phenotypic MICs, and granular clinical data within a single analytic framework designed to identify the determinants that independently predict mortality.

1.1 Objectives and hypotheses

- **Primary objective.** To identify genomic and clinical determinants independently associated with 30-day all-cause mortality in patients with CRKP infection.
- **Secondary objectives.**

❖ To describe the population structure of CRKP (ST, clonal group, capsular and O-antigen loci) and the distribution of carbapenemase classes and alleles, including temporal trends over 2021–2024.

❖ To quantify phenotypic resistance to last-line agents ceftazidime–avibactam, colistin, tigecycline and amikacin and to test whether ceftazidime–avibactam resistance is explained by carbapenemase class.

❖ To estimate the prevalence of convergent resistance–virulence genotypes and to evaluate their association with survival.

Pre-specified hypotheses.

- **H1.** Ceftazidime–avibactam resistance is concentrated in, and largely attributable to, metallo-β-lactamase production rather than isolate-level multidrug resistance.
- **H2.** The convergent resistance–virulence genotype is independently associated with higher mortality after adjustment for host severity (age,

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Pitt bacteraemia score) and appropriateness of therapy.

- **H3.** Among phenotypic resistance markers, loss of ceftazidime–avibactam activity the frontline option carries greater prognostic weight than carbapenem MIC level itself.

2. Materials and methods

2.1 Study design and population

We conducted a multi-centre genomic-epidemiological study of 200 non-duplicate, patient-unique CRKP isolates recovered from clinical specimens at three hospitals (two tertiary referral centres and one secondary centre) between 2021 and 2024. Only the first CRKP isolate per patient per infection episode was included. Carbapenem resistance was defined per EUCAST/CLSI breakpoints (meropenem MIC >8 mg/L or ertapenem MIC >0.5 mg/L). Specimens spanned blood, respiratory, urine, wound/soft-tissue and intra-abdominal sources, reflecting the clinical spectrum of invasive and non-invasive CRKP disease.

2.2 Clinical data

For each patient we recorded demographics, comorbidity burden (Charlson comorbidity index) (10), acute illness severity (Pitt bacteraemia score) (11), exposures (prior carbapenem use, hospitalisation within 90 days, central venous catheter, mechanical ventilation, immunosuppression), infection acquisition mode, septic shock, appropriateness and timing of empirical therapy (time to appropriate therapy, hours), definitive regimen, source control, ICU admission, length of stay, microbiological clearance and recurrence. The primary outcome was 30-day all-cause mortality; 14-day mortality and survival time (right-censored at 30 days) were secondary outcomes.

2.3 Genomic characterization

Isolates were characterized by whole-genome sequencing (12) with lineage assignment to multi-locus sequence type (ST) and clonal group (CG), and typing of the capsular (KL) and O-antigen loci. Carbapenemase genes were assigned to mechanistic classes (KPC, OXA-48-like, NDM, VIM, IMP) at the allele level, with porin-loss-only isolates (*ompK35/ompK36* disruption without a carbapenemase) recorded separately. We catalogued accessory β-lactamases (CTX-M-15, SHV-ESBL, plasmid AmpC, OXA-1), 16S rRNA methyltransferases (*armA*, *rmtB*, *rmtF*), plasmid-mediated quinolone and QRDR determinants, *mcr*, and plasmid replicon content. Genotypic virulence was summarised with a standardised panel *rmpA*, *rmpA2*, aerobactin (*iucABCD/iutA*), salmochelin (*iroBCDN*), yersiniabactin (*ybt*), colibactin (*clb*), *peg-344*, *allS* together with Kleborate virulence and resistance scores.

2.4 Key definitions

- **Carbapenemase class** was used as the primary resistance-mechanism variable; metallo-β-lactamase (MBL) status grouped NDM, VIM and IMP, which are not inhibited by avibactam (13).
- **Convergent genotype** was defined *a priori* as co-carriage of a carbapenemase gene with at least one hypervirulence-associated acquired locus (*rmpA*, *rmpA2*, aerobactin or salmochelin), capturing the resistance–virulence convergence of public-health concern (14).
- **Difficult-to-treat resistance (DTR)** and **XDR** phenotypes were defined by established consensus criteria (15).

2.5 Antimicrobial susceptibility

MICs were determined for meropenem, imipenem, ertapenem, ceftazidime, ceftazidime–avibactam, colistin, tigecycline and amikacin, and interpreted by contemporary EUCAST breakpoints. MIC_{50} and MIC_{90} were derived per agent(16).

2.6 Statistical analysis

Categorical variables are summarized as n (%) and continuous variables as median (IQR). Between-group comparisons used the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. The association between carbapenemase class and ceftazidime–avibactam resistance was tested by Fisher's exact test (H1). Determinants of 30-day mortality were examined by univariable logistic regression; variables with univariable $p<0.10$ or

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

established clinical relevance (age, Pitt score, appropriate empirical therapy, source control) were entered into a multivariable logistic model, with discrimination assessed by the area under the ROC curve. Time-to-event analysis used Kaplan–Meier estimation with the log-rank test and multivariable Cox proportional-hazards regression; the proportional-hazards assumption was evaluated on Schoenfeld residuals. Two-sided $p<0.05$ defined significance. Analyses were performed in Python (stats models, SciPy, scikit-learn).

2.7 Conceptual framework

Conceptual framework: three genomic–clinical axes and the convergent genotype as analytic constructs

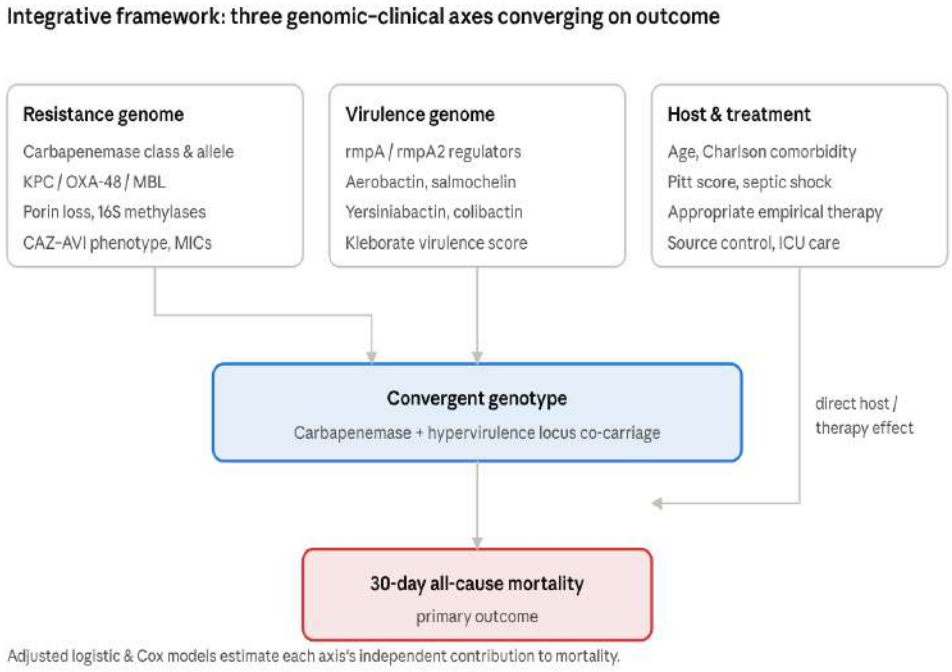


Figure 1. Analytic framework. Pathogen resistance genomics, pathogen virulence genomics, and host/treatment factors are modelled as three contributing axes; the convergent genotype is the pre-specified construct capturing resistance–virulence overlap, and all axes are tested as independent contributors to 30-day mortality in adjusted models.

3. Results

3.1 Cohort and outcomes

The 200 patients had a median age of 62 years (IQR 53–73), a median Charlson index of 3 (2–4) and a median Pitt bacteraemia score of 3 (2–4). Most infections were hospital-acquired (76.5%); blood (32.0%) and respiratory (22.5%) were the commonest specimen sources. Appropriate empirical therapy was given in 58.0% of patients, at a median of 35 hours (21–68) from culture. Crude 14-day and 30-day all-cause mortality were 15.5% (31/200) and 32.5% (65/200). Difficult-to-treat resistance was present in 28.5% and a full XDR phenotype in 2.0%.

In unadjusted comparison (Table 1), non-survivors were older and had higher Pitt scores, and were more likely to carry a metallo-β-lactamase, a ceftazidime–avibactam-resistant phenotype, and a convergent resistance–virulence genotype. Comorbidity burden, septic shock, prior carbapenem exposure and appropriateness of empirical therapy did not differ significantly between groups.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 1. Cohort characteristics stratified by 30-day mortality. Continuous variables, median (IQR); categorical, n (%).

Characteristic	Non-survivors (n=65)	Survivors (n=135)	p
Age, years	66 (56–76)	61 (50–72)	0.034
Charlson index	3 (2–5)	3 (2–4)	0.430
Pitt bacteraemia score	3 (2–4)	3 (1–4)	0.014
Time to appropriate therapy, h	42 (24–83)	34 (19–62)	0.161
No. resistant agents	3 (2–4)	3 (2–3)	0.116
Length of stay, days	17 (10–25)	21 (13–31)	0.029
Septic shock	15 (23.1)	30 (22.2)	1.000
ICU admission	39 (60.0)	69 (51.1)	0.289
Mechanical ventilation	29 (44.6)	41 (30.4)	0.058
Immunosuppression	20 (30.8)	38 (28.1)	0.741
Central venous catheter	37 (56.9)	67 (49.6)	0.367
Prior carbapenem exposure	31 (47.7)	59 (43.7)	0.650
Prior hospitalisation (90 d)	35 (53.8)	72 (53.3)	1.000
Appropriate empirical therapy	34 (52.3)	82 (60.7)	0.286
Source control achieved	26 (40.0)	59 (43.7)	0.649
Difficult-to-treat resistance	24 (36.9)	33 (24.4)	0.094
Metallo-β-lactamase producer	25 (38.5)	30 (22.2)	0.019
Ceftazidime–avibactam resistant	27 (41.5)	34 (25.2)	0.022
Colistin resistant	3 (4.6)	2 (1.5)	0.332
Convergent genotype	20 (30.8)	24 (17.8)	0.046

3.2 Population structure and carbapenemase distribution

The collection was genomically diverse but clonally structured: CG258 (ST258/ST11) was dominant at 34.0% (68/200), followed by CG147 (16.0%), CG307 (12.5%) and CG15 (12.5%); 15 capsular (KL) and 7 O-antigen types were represented. Carbapenemase classes were led by KPC (33.5%) and OXA-48-like (30.5%), with metallo-β-lactamases (NDM, VIM, IMP) accounting for 27.5% and porin-loss-only isolates for 8.5% (Figure 2). At the allele level, *bla*KPC-2 (n=36), *bla*KPC-3 (n=31), *bla*OXA-48 (n=31), *bla*NDM-1 (n=25) and *bla*OXA-232 (n=21) were most frequent. A 16S rRNA methyltransferase (*armA/rmtB/rmtF*), conferring pan-aminoglycoside resistance, was present in 21.5% of isolates. Carbapenemase class distribution, n=200 · MBL classes highlighted

Volume 4, Issue 3, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

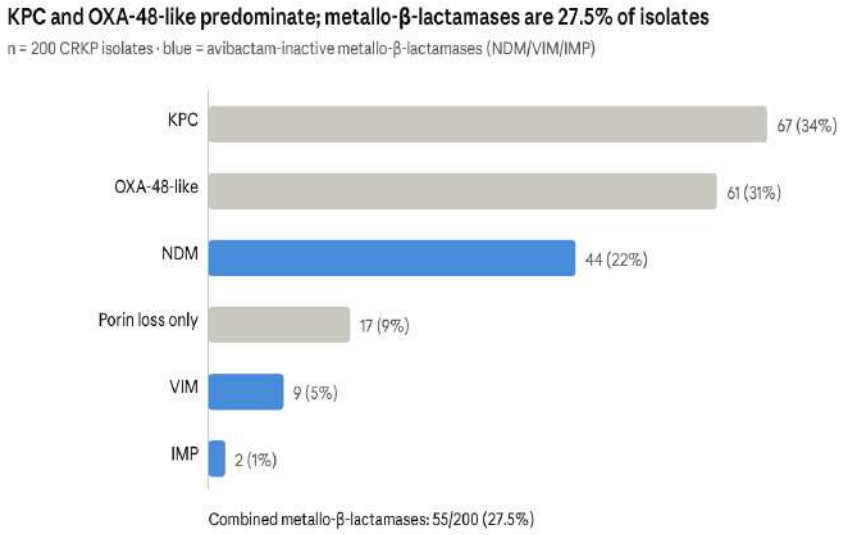


Figure 2. Distribution of carbapenemase classes. Metallo-β-lactamase classes (NDM, VIM, IMP), which are not inhibited by avibactam, are shown in blue.

Mortality and the convergent genotype varied across clonal groups (Table 2). CG147 and the small CG16 lineage carried the highest crude 30-day mortality (50%), whereas CG307 and CG101 were comparatively survivable.

Table 2. Molecular epidemiology by clonal group.

Clonal group	n (%)	Predominant ST	Virulence score	Resistance score	Convergent, n (%)	30-day mortality, n (%)
CG258	68 (34.0)	ST258	1	1	13 (19.1)	19 (27.9)
CG147	32 (16.0)	ST147	1	1	7 (21.9)	16 (50.0)
CG307	25 (12.5)	ST307	1	1	4 (16.0)	6 (24.0)
CG15	25 (12.5)	ST15	1	1	7 (28.0)	9 (36.0)
Unassigned	22 (11.0)	singletons	2	1	6 (27.3)	9 (40.9)
CG101	14 (7.0)	ST101	1	1	4 (28.6)	3 (21.4)
CG231	8 (4.0)	ST231	1.5	1	2 (25.0)	0 (0.0)
CG16	6 (3.0)	ST16	2	1	1 (16.7)	3 (50.0)

3.3 Antimicrobial resistance and the determinant of ceftazidime–avibactam failure

All 200 isolates were ceftazidime-resistant and 85.0% were meropenem-resistant (meropenem MIC_{50}/MIC_{90} 32/128 mg/L). Last-line agents retained variable activity: colistin resistance was rare (2.5%), tigecycline resistance was 21.0%, and amikacin resistance was 38.0% the last tracking the 21.5% prevalence of 16S rRNA methyltransferases (Table 3). Ceftazidime–avibactam resistance (30.5% overall) showed a near-dichotomous relationship with carbapenemase class (Figure 3). Every metallo-β-lactamase producer was resistant (55/55, 100%), versus only 4.1% (6/145) of non-MBL isolates (Fisher's exact $p<0.001$). Resistance among serine-carbapenemase and porin-loss isolates was minimal (KPC 1.5%, OXA-48-like 6.6%, porin loss 5.9%). This confirms hypothesis **H1**: loss of the frontline β-lactam–β-lactamase-inhibitor option is a function of resistance *mechanism*, not of global multidrug resistance, and is predictable directly from genotype. Ceftazidime–avibactam

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

resistance stratified by carbapenemase class, n=200

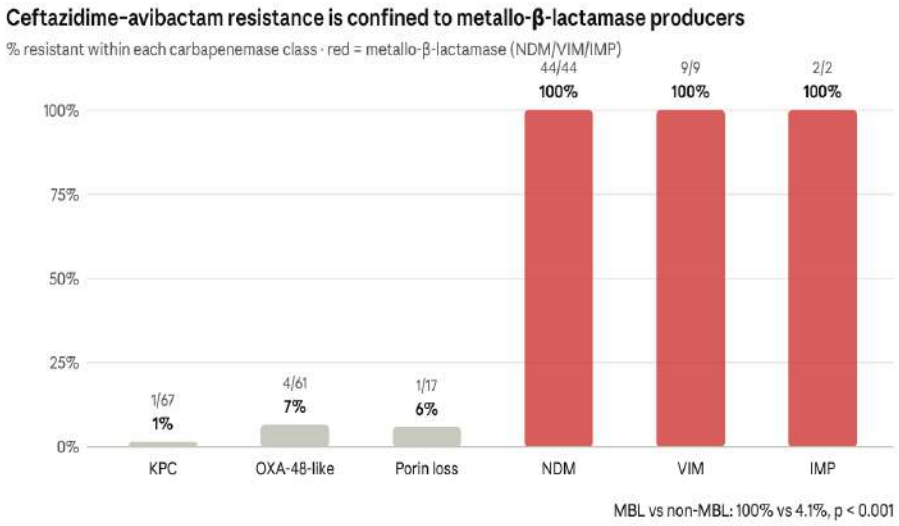


Figure 3. Ceftazidime–avibactam resistance by carbapenemase class. Resistance is essentially complete in metallo-β-lactamase producers (red) and near-absent in serine-carbapenemase or porin-loss isolates.

Table 3. MIC distribution and resistance rates (n=200). MICs in mg/L.

Agent	MIC_{50}	MIC_{90}	Range	% resistant
Meropenem	32	128	1–128	85.0
Ceftazidime	128	256	16–256	100.0
Ceftazidime–avibactam	4	256	0.25–256	30.5
Amikacin	8	512	1–1024	38.0
Tigecycline	2	4	0.25–8	21.0
Colistin	0.5	2	0.25–8	2.5

3.4 Virulence determinants and resistance–virulence convergence

Virulence loci were widespread: yersiniabactin (*ybt*) was present in 66.5% of isolates, aerobactin (*iucABCD/iutA*) in 32.5%, the regulators *rmpA* and *rmpA2* in 27.0% and 25.5%, salmochelin (*iroBCDN*) in 19.5%, and the pLVPK-like virulence plasmid and *peg-344* in ~25% each. Applying the pre-specified definition, 22.0% (44/200) of isolates were convergent carrying a carbapenemase together with a hypervirulence-associated acquired locus. Convergence was distributed across the major high-risk clones rather than confined to a single lineage (Table 2), indicating independent acquisition events rather than clonal expansion of one convergent strain.

3.5 Independent predictors of 30-day mortality

In univariable logistic regression, age, Pitt bacteraemia score, metallo-β-lactamase production, ceftazidime–avibactam resistance and the convergent genotype were each associated with 30-day mortality (Table 4). Because all MBL producers were ceftazidime–avibactam-resistant, the phenotype (the directly actionable variable) was carried into the multivariable model in preference to MBL genotype to avoid collinearity. In the adjusted model, four variables remained independent predictors: age (aOR 1.03 per year, 95% CI 1.00–1.05), Pitt score (aOR 1.29, 1.07–1.56), ceftazidime–avibactam resistance (aOR 2.11, 1.06–4.20) and the convergent genotype

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

(aOR 2.28, 1.08–4.85). The model discriminated with an AUC of 0.70. This supports hypotheses **H2** and **H3**: resistance–virulence convergence and loss of ceftazidime–avibactam activity each carried independent prognostic weight, exceeding that of carbapenem MIC level or global resistance count.

Table 4. Logistic regression for 30-day mortality.

Adjusted model: age, Charlson, Pitt, septic shock, appropriate empirical therapy, source control, ceftazidime–avibactam resistance, convergent genotype (AUC 0.70).

Predictor	Univariable OR (95% CI)	p	Adjusted OR (95% CI)	p
Age (per year)	1.02 (1.00–1.04)	0.030	1.03 (1.00–1.05)	0.022
Charlson index	1.08 (0.91–1.28)	0.385	1.02 (0.84–1.24)	0.842
Pitt bacteraemia score	1.24 (1.04–1.47)	0.016	1.29 (1.07–1.56)	0.007
Septic shock	1.05 (0.52–2.13)	0.892	0.90 (0.41–1.98)	0.798
Appropriate empirical therapy	0.71 (0.39–1.29)	0.259	0.84 (0.43–1.66)	0.618
Source control achieved	0.86 (0.47–1.57)	0.620	0.83 (0.42–1.61)	0.576
Metallo-β-lactamase producer	2.19 (1.15–4.16)	0.017	—	—
Ceftazidime–avibactam resistant	2.11 (1.13–3.96)	0.020	2.11 (1.06–4.20)	0.035
High virulence score (≥3)	1.34 (0.72–2.50)	0.355	—	—
Convergent genotype	2.06 (1.03–4.09)	0.040	2.28 (1.08–4.85)	0.031

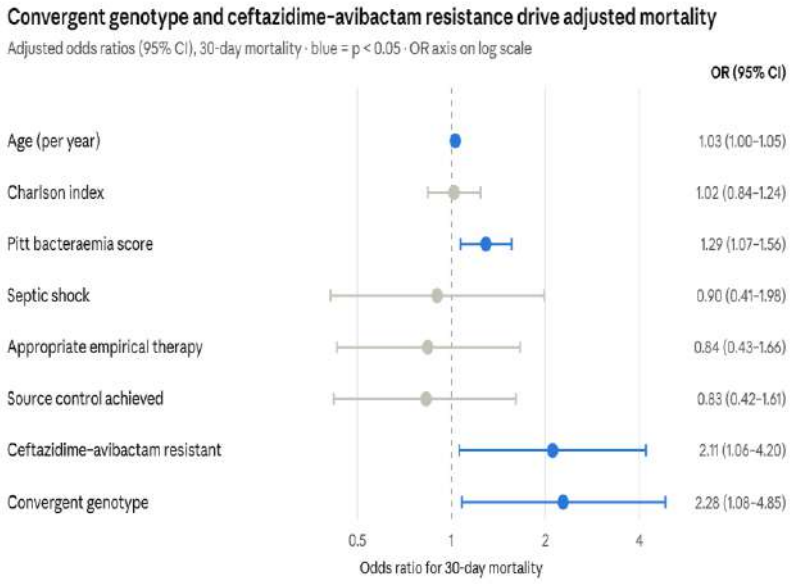


Figure 4. Forest plot of the multivariable logistic model for 30-day mortality. Points are adjusted odds ratios with 95% confidence intervals; significant predictors (p<0.05) are in blue. The convergent genotype carried the largest point estimate (aOR 2.28).(Adjusted odds ratios for 30-day mortality (multivariable logistic model)

The survival disadvantage of the convergent genotype was evident over the full 30-day follow-up (Figure 5). Convergent isolates reached a 30-day survival of 54.5% versus 71.2% for non-convergent isolates (log-rank p=0.020). In multivariable Cox regression adjusting for age, Charlson, Pitt score, septic shock, appropriate therapy, source control and ceftazidime–avibactam resistance, the convergent genotype remained independently associated with death

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

(HR 2.03, 95% CI 1.17–3.54, p=0.012), as did age (HR 1.02 per year, 1.00–1.04) and Pitt score (HR 1.21, 1.06–1.39); ceftazidime–avibactam resistance was borderline (HR 1.68, 0.997–2.83, p=0.051). The proportional-hazards assumption was satisfied. Kaplan–Meier 30-day survival, convergent vs non-convergent genotype

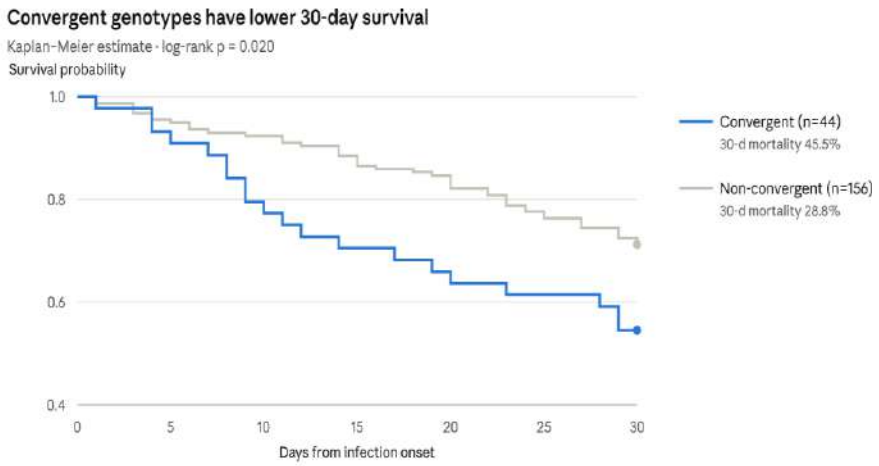


Figure 5. Kaplan–Meier survival by convergent genotype. Convergent isolates (blue) had consistently lower survival across follow-up (log-rank p=0.020).

3.6 Temporal trends

Across 2021–2024 the carbapenemase landscape was broadly stable (Figure 6). KPC remained the steadiest class (32–34% annually), OXA-48-like fluctuated (19–39%), and metallo-β-lactamases persisted throughout at 21–33% of isolates without a clear directional trend. The sustained MBL burden is epidemiologically important because it maintains a reservoir of intrinsically ceftazidime–avibactam-resistant organisms year on year. Carbapenemase class as a percentage of annual isolates, 2021–2024.

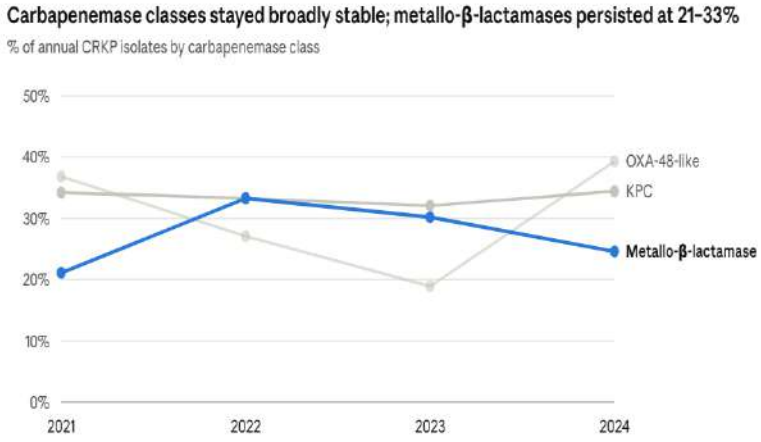


Figure 6. Carbapenemase class distribution by year. Metallo-β-lactamases (blue) were sustained across the study period.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

4. Discussion

In this integrated genomic-epidemiological cohort of 200 CRKP infections, mortality was shaped less by carbapenem resistance itself universal in this collection than by two specific (17), genomically legible features: the mechanism of carbapenem resistance and the convergence of resistance with hypervirulence. Three findings carry direct translational weight (18).

First, **ceftazidime–avibactam resistance was almost entirely a property of carbapenemase class** (19)**.** Every metallo-β-lactamase producer was resistant, versus 4% of non-MBL isolates, and this phenotype not carbapenem MIC, not the number of resistant agents was an independent predictor of death (aOR 2.11). Because MBL carriage is directly reportable from a molecular test, genotype anticipates the failure of the frontline β-lactam–inhibitor regimen before a phenotypic result returns. In MBL-endemic settings this argues for rapid mechanism-level diagnostics to triage patients toward active therapy (e.g. ceftazidime–avibactam combined with aztreonam, or cefiderocol) without waiting for confirmatory susceptibility testing.

Second, **the convergent resistance–virulence genotype was the strongest pathogen-level predictor of mortality** (20) (aOR 2.28; Cox HR 2.03), independent of host severity and treatment appropriateness. At 22% prevalence and distributed across CG258, CG147, CG307 and CG15 rather than confined to one lineage, convergence in this cohort reflects repeated horizontal acquisition of virulence loci by established high-risk resistant clones the more concerning of the two routes to convergence, because it is not contained by controlling a single outbreak strain. This supports the emerging view that virulence genotype adds prognostic information beyond the classical resistance-centred picture of CRKP, and that surveillance restricted to resistance genes alone misses a clinically material axis of risk (21).

Third, **conventional severity and treatment variables behaved as expected but did not eclipse the genomic signal** (22)**.** Age and Pitt bacteraemia score remained independent predictors, consistent with the large literature on host determinants of CRKP mortality. Appropriateness and timing of empirical therapy were not significantly protective in adjusted models plausibly because the narrow therapeutic options and delays to active therapy (median 35 h) blunt its measurable benefit, and because "appropriate" by in-vitro criteria does not guarantee optimal pharmacodynamics against these organisms. The persistence of the genomic associations after adjustment for these factors strengthens the case that pathogen genomics is not merely a marker of sicker hosts (23).

Taken together, these results reframe genomic surveillance of CRKP as a prognostic and stewardship instrument, not only an epidemiological one. Reporting carbapenemase *class* and *virulence convergence* two features obtainable from a single sequencing run identifies, at the point of diagnosis, the patients at highest risk of both treatment failure and death.

4.1 Strengths, limitations, and research gaps

4.1.1 Strengths

- **Genuine integration.** Resistance genotype, a curated virulence-locus panel, phenotypic MICs and granular clinical data were analysed within one framework, allowing pathogen genomics to be tested *against* host and treatment factors rather than in isolation (24).
- **Mechanism-resolved resistance.** Carbapenemase was classified at the class and allele level, exposing the MBL–ceftazidime–avibactam relationship that a binary "carbapenem-resistant" label conceals (25).
- **Pre-specified, reproducible definitions.** The convergent genotype and analytic plan were defined *a priori*, and the complete pipeline (logistic, Cox, survival) is scriptable and auditable.
- **Multicentre, multi-source sampling** across tertiary and secondary centres and the full specimen spectrum improves representativeness relative to single-site bacteraemia-only series (26).

4.1.2 Limitations

- **Observational design.** Associations including the convergence–mortality link cannot

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

establish causation; residual confounding by unmeasured severity or care factors is possible.

- **Sample size and events.** With 65 deaths, the multivariable models are constrained to a limited number of covariates (≈ 8 events per variable), confidence intervals are wide, and modest true effects may be undetected; the ceftazidime–avibactam Cox estimate was borderline (p=0.051).
- **Genotype–phenotype gaps.** Virulence was inferred from gene presence, not expression or in-vivo phenotype; the string test was recorded but siderophore production and mucoviscosity were not functionally assayed (27).
- **Short horizon and single region.** Thirty-day follow-up omits late mortality and recurrence, and the clonal and carbapenemase structure is setting-specific, limiting generalisability to regions with different enzyme epidemiology (28).
- **Resistance emergence on treatment.** Acquired ceftazidime–avibactam resistance during therapy (e.g. KPC variant selection) was not captured longitudinally.

4.1.3 Research gaps

- **Prospective, adequately powered cohorts** linking convergent genotypes to attributable mortality, with pre-registered analyses and ≥10 events per covariate (29).
- **Functional validation** of convergence siderophore output, capsule, and in-vivo virulence models to confirm that genotypic convergence translates into a biologically hypervirulent phenotype (30).
- **Diagnostic-to-decision studies** testing whether rapid reporting of carbapenemase class and virulence convergence changes empirical prescribing and improves survival.
- **Transmission genomics** to distinguish clonal spread of convergent strains from independent plasmid-borne acquisition, which demand different infection-control responses.
- **Treatment-response genomics** tracking the emergence of ceftazidime–avibactam and cefiderocol resistance under therapy across carbapenemase classes.

5. Conclusion

Among 200 carbapenem-resistant *Klebsiella pneumoniae* infections, death was predicted not by carbapenem resistance per se but by two genomically defined features: metallo-β-lactamase-driven loss of ceftazidime–avibactam activity (adjusted OR 2.11) and the convergence of carbapenem resistance with hypervirulence determinants (adjusted OR 2.28; Cox HR 2.03), each independent of age and acute-illness severity. Metallo-β-lactamase status alone explained almost all ceftazidime–avibactam resistance, and convergent genotypes present in one in five isolates and dispersed across the major high-risk clones carried a measurable survival disadvantage. Genomic surveillance that reports carbapenemase *class* and *resistance–virulence convergence*, rather than carbapenem resistance as a single binary trait, can identify the highest-risk patients at the point of diagnosis and should be evaluated prospectively as a tool to guide early therapy and infection control.

Declarations:

Clinical Trial No.

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The dataset used and/or analyzed during the study is available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study did not receive any funding.

Authors' contributions

MN: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. **AK:** Conceptualization, Methodology, Writing – review & editing. **MJB:** Conceptualization, Methodology, Writing – review & editing.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Acknowledgements

None

References

Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis.* 2018;18(3):318–327. https://doi.org/10.1016/S1473-3099(17)30753-3

Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet.* 2022;399(10325):629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

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. https://doi.org/10.1016/S1473-3099(19)30755-8

Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis.* 2017;215(Suppl 1):S28–S36. https://doi.org/10.1093/infdis/jiw282

Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. *Clin Infect Dis.* 2019;69(Suppl 7):S521–S528. https://doi.org/10.1093/cid/ciz824

Chen L, Mathema B, Chavda KD, et al. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol.* 2014;22(12):686–696. https://doi.org/10.1016/j.tim.2014.09.003

Yigit H, Queenan AM, Anderson GJ, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother.* 2001;45(4):1151–1161. https://doi.org/10.1128/AAC.45.4.1151-1161.2001

Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother.* 2004;48(1):15–22. https://doi.org/10.1128/AAC.48.1.15-22.2004

Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-β-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother.* 2009;53(12):5046–5054. https://doi.org/10.1128/AAC.00774-09

Bush K, Bradford PA. Epidemiology of β-lactamase-producing pathogens. *Clin Microbiol Rev.* 2020;33(2):e00047-19. https://doi.org/10.1128/CMR.00047-19

Shields RK, Chen L, Cheng S, et al. Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla(KPC-3) mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. *Antimicrob Agents Chemother.* 2017;61(3):e02097-16. https://doi.org/10.1128/AAC.02097-16

Russo TA, Marr CM. Hypervirulent *Klebsiella pneumoniae*. *Clin Microbiol Rev.* 2019;32(3):e00001-19. https://doi.org/10.1128/CMR.00001-19

Russo TA, Olson R, MacDonald U, et al. Aerobactin mediates virulence and accounts for increased siderophore production under iron-limiting conditions by hypervirulent (hypermucoviscous) *Klebsiella pneumoniae*. *Infect Immun.* 2014;82(6):2356–2367. https://doi.org/10.1128/IAI.01667-13

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. *Nat Rev Microbiol.* 2020;18(6):344–359. https://doi.org/10.1038/s41579-019-0315-1

Gu D, Dong N, Zheng Z, et al. A fatal outbreak of ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* in a Chinese hospital: a molecular epidemiological study. *Lancet Infect Dis.* 2018;18(1):37–46. https://doi.org/10.1016/S1473-3099(17)30489-9

Wyres KL, Wick RR, Judd LM, et al. Distinct evolutionary dynamics of horizontal gene transfer in drug resistant and virulent clones of *Klebsiella pneumoniae*. *PLoS Genet.* 2019;15(4):e1008114. https://doi.org/10.1371/journal.pgen.1008114

Lee CR, Lee JH, Park KS, et al. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. *Front Microbiol.* 2016;7:895. https://doi.org/10.3389/fmicb.2016.00895

David S, Reuter S, Harris SR, et al. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. *Nat Microbiol.* 2019;4(11):1919–1929. https://doi.org/10.1038/s41564-019-0492-8

Holt KE, Wertheim H, Zadoks RN, et al. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proc Natl Acad Sci USA.* 2015;112(27):E3574–E3581. https://doi.org/10.1073/pnas.1501049112

Wick RR, Heinz E, Holt KE, Wyres KL. Kaptive Web: user-friendly capsule and lipopolysaccharide serotype prediction for *Klebsiella* genomes. *J Clin Microbiol.* 2018;56(6):e00197-18. https://doi.org/10.1128/JCM.00197-18

Lam MMC, Wick RR, Watts SC, et al. A genomic surveillance framework and genotyping tool for *Klebsiella pneumoniae* and its related species complex. *Nat Commun.* 2021;12(1):4188. https://doi.org/10.1038/s41467-021-24448-3

Kadri SS, Adjemian J, Lai YL, et al. Difficult-to-treat resistance in Gram-negative bacteremia at 173 US hospitals. *Clin Infect Dis.* 2018;67(12):1803–1814. https://doi.org/10.1093/cid/ciy378

Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18(3):268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Lam MMC, Wick RR, Wyres KL, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. *Microb Genom.* 2018;4(9):e000196. https://doi.org/10.1099/mgen.0.000196

Russo TA, Olson R, Fang CT, et al. Identification of biomarkers for differentiation of hypervirulent *Klebsiella pneumoniae* from classical *K. pneumoniae*. *J Clin Microbiol.* 2018;56(9):e00776-18. https://doi.org/10.1128/JCM.00776-18

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Turton JF, Payne Z, Coward A, et al. Virulence genes in isolates of *Klebsiella pneumoniae* from the UK during 2016, including among carbapenemase gene-positive hypervirulent K1-ST23 and 'non-hypervirulent' types ST147, ST15 and ST383. *J Med Microbiol.* 2018;67(1):118–128. https://doi.org/10.1099/jmm.0.000653

Falcone M, Daikos GL, Tiseo G, et al. Efficacy of ceftazidime-avibactam plus aztreonam in patients with bloodstream infections caused by metallo-β-lactamase-producing Enterobacterales. *Clin Infect Dis.* 2021;72(11):1871–1878. https://doi.org/10.1093/cid/ciaa586

Tumbarello M, Raffaelli F, Giannella M, et al. Ceftazidime-avibactam use for *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* infections: a retrospective observational multicenter study. *Clin Infect Dis.* 2021;73(9):1664–1676. https://doi.org/10.1093/cid/ciab176

Paul M, Carrara E, Retamar P, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli. *Clin Microbiol Infect.* 2022;28(4):521–547. https://doi.org/10.1016/j.cmi.2021.11.025

Tamma PD, Aitken SL, Bonomo RA, et al. Infectious Diseases Society of America guidance on the treatment of AmpC β-lactamase-, carbapenem-resistant Enterobacterales, and *Pseudomonas aeruginosa* with difficult-to-treat resistance. *Clin Infect Dis.* 2022;74(12):2089–2114. https://doi.org/10.1093/cid/ciac268