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Clonal OmpK35 truncation and incomplete genotype-phenotype correlation underlie cefiderocol resistance in carbapenem-resistant, hypervirulent Klebsiella pneumoniae from Romania

Anurag Kumar Bari
University of Groningen, University Medical Center Groningen
Mihai Octavian Dan
Carol Davila University of Medicine and Pharmacy
Dominique Snel
University Medical Center Groningen
Alexandra Cireșă
Carol Davila University of Medicine and Pharmacy
Alexandru Rafila
Prof. Dr. Matei Balș National Institute of Infectious Diseases, Bucharest, Romania
Daniela Tălăpan
Carol Davila University of Medicine and Pharmacy
Bhanu Sinha
University of Groningen, University Medical Center Groningen
John W.A. Rossen
University of Groningen, University Medical Center Groningen
Basil Britto Xavier
b.b.xavier@umcg.nl

University of Groningen, University Medical Center Groningen

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Abstract

Purpose.

Convergence of carbapenem resistance and hypervirulence in *Klebsiella pneumoniae* is an emerging global threat, historically described almost exclusively in hospital inpatients. We characterised the genomic epidemiology, resistance determinants and cefiderocol-resistance mechanisms of *K. pneumoniae* isolated from patients with documented prior hospital exposure, including outpatients, at a Romanian tertiary-care institution.

Methods.

Twenty-five clinical *K. pneumoniae* isolates collected between August 2023 and July 2025 underwent long-read (Oxford Nanopore) whole-genome sequencing, Kleborate-based typing and resistance/virulence scoring, average nucleotide identity (ANI)-based clonality analysis, core-genome SNP comparison against a concurrent Romanian inpatient collection, and targeted BLAST-based screening of the porins OmpK35/OmpK36 and the siderophore receptors CirA/FyuA for loss-of-function variation underlying cefiderocol resistance.

Results.

Kleborate resolved seven sequence types; ST383 (n = 12) and ST101 (n = 8) accounted for 80% of isolates. NDM (n = 16: NDM-5, n = 10, ST383-restricted; NDM-1, n = 6) and OXA-48 (n = 11) were the dominant carbapenemases; nine isolates co-carried an NDM variant with OXA-48. Eight ST383 isolates carried the aerobactin locus (*iuc*) and scored 3 on the Kleborate virulence scale, meeting criteria for convergent carbapenem-resistant hypervirulent *K. pneumoniae* (CR-hvKp); all eight also carried *armA*, a 16S rRNA methyltransferase conferring pan-aminoglycoside resistance, absent from all 17 aerobactin-negative isolates. All 25 isolates were cefiderocol-resistant by EUCAST criteria (MIC 4–32 mg/L) yet remained susceptible to aztreonam-avibactam. NDM carriage and OmpK35 truncation each trended toward higher cefiderocol MIC, but genotype did not predict MIC deterministically: isolates genotypically identical at every screened locus differed up to four-fold in MIC. The OmpK35 truncation was clonally inherited across all eight ST101 isolates but arose independently at least twice within ST383. Immune-chromatographic carbapenemase testing was discordant with WGS in 4/25 (16%) isolates. Core-SNP analysis against a concurrent 101-isolate inpatient collection identified seven ST383 pairs within a 20-SNP transmission threshold.

Conclusions.

A convergent, cefiderocol-resistant CR-hvKp ST383 lineage circulates across outpatient and inpatient settings in Romania, with clonal aminoglycoside co-resistance and heterogeneous, only partially genotype-predictable cefiderocol resistance. These findings support extending genomic surveillance beyond acute inpatient care.

Introduction

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is a World Health Organization critical-priority pathogen, most often acquired in healthcare settings; CRKP detected outside the hospital in Europe has generally been linked to recent healthcare contact, travel, or gastrointestinal carriage [1, 2]. In parallel, hypervirulent *K. pneumoniae* (hvKp), historically a community pathogen causing pyogenic liver abscess in classical K1/K2 lineages, has evolved into convergent CR-hvKp strains in which high-risk clones acquire both carbapenemase-encoding plasmids and virulence plasmids carrying the aerobactin (*iuc*) locus [2, 3]. Among convergent lineages, ST383 carrying *bla*NDM together with *iuc* on hybrid virulence–resistance plasmids has been repeatedly reported from Eastern Europe and the Balkans, predominantly in hospital inpatients [3, 4].

Cefiderocol, a siderophore-conjugated cephalosporin that exploits bacterial iron-uptake pathways for periplasmic entry, is a last-resort agent for carbapenem-resistant Gram-negative infections. Resistance can arise through carbapenemase-mediated hydrolysis (particularly by metallo-β-lactamases such as NDM), through loss-of-function mutation in the porins OmpK35/OmpK36 that provide the drug's secondary diffusion route, or through mutation of the TonB-dependent catecholate siderophore receptors (e.g. *CirA*) that mediate its primary active-uptake route. The relative contribution and clonal versus convergent-evolutionary origin of these mechanisms in circulating clinical populations remains incompletely characterised, particularly in South-Eastern Europe.

Romanian data on CRKP are sparse and have largely concerned hospital inpatients, since surveillance of isolates from outpatients is limited. We report a genomic characterisation of 25 clinical *K. pneumoniae* isolates, all from patients with documented prior hospital exposure (including outpatients, general-ward and intensive-care patients), collected at a single Romanian tertiary-care institution between August 2023 and July 2025. Our aims were (i) to characterise the clonal structure of the isolates, (ii) to map the distribution of carbapenemase-encoding genes and their likely plasmid vehicles, (iii) to quantify the extent of resistance–virulence convergence, and (iv) to resolve the genomic mechanisms underlying cefiderocol resistance and test whether genotype predicts phenotype in this population.

Materials and methods

Isolates and clinical microbiology. The 25 clinical *K. pneumoniae* isolates (isolate IDs 01–94) were recovered from patients admitted to the 'Prof. Dr. Matei Balș' National Institute of Infectious Diseases, Bucharest, Romania, with suspected infection, between August 2023 and July 2025. Specimens were inoculated on Columbia sheep blood agar, chocolate agar with Polivitex, and lactose agar

(ThermoScientific–Oxoid, Wesel, Germany; bioMérieux, Marcy-l'Étoile, France), incubated 18–24 h at 37°C aerobically, and identified by MALDI-TOF MS (Biotyper, Bruker Daltonik, Bremen, Germany; software v4.1).

Antimicrobial susceptibility testing. Susceptibility testing used MICRONAUT-S Gram-negative (customised plate, Romania GN 2 EUCAST; Bruker Daltonik), interpreted per EUCAST guidelines v15.0. Isolates with meropenem MIC > 0.125 µg/mL underwent ESBL/carbapenemase lateral-flow testing (NG-Test CTX-M Multi, NG Biotech, France; RESIST-3 O.K.N., Coris BioConcept, Belgium, resolving OXA-48-, KPC- and NDM-type carbapenemases on three lines). Cefiderocol susceptibility was tested by UMIC® (Bruker Daltonik) and interpreted per EUCAST v14.0 (S ≤ 2 mg/L, R > 2 mg/L). MDR/XDR/PDR classification followed international consensus definitions [9].

Whole-genome sequencing and assembly. Genomic DNA was extracted with MagAttract (Qiagen) and sequenced by Oxford Nanopore long-read sequencing (Rapid Barcoding SQK-RBK114.24, R10.4.1 flowcells; Dorado basecalling; NanoPlot QC). Assembly used Flye v2.9.1; assembly quality was assessed with QUAST v5.2.0 and Bandage v0.9.0.

Genotyping and comparative genomics. Species confirmation, MLST, K/O-locus typing (Kaptive), yersiniabactin/aerobactin/colibactin locus typing, resistance determinants, and Kleborate virulence (0–5) and resistance (0–3) scores were obtained with Kleborate v3.2.4 (Kp-complex pathway) [5]. Acquired resistance genes, virulence genes and plasmid replicons were additionally screened with abricate v1.0 against CARD, ResFinder, VFDB and PlasmidFinder (September 2025). Because acquired-gene databases do not screen for the chromosomal loss-of-function mutations that also drive cefiderocol resistance, the porins OmpK35 and OmpK36 and the siderophore receptors CirA and FyuA were additionally screened by tblastn (BLAST+ v2.x) against curated *K. pneumoniae* RefSeq proteins (OmpK35, WP_499823533.1; OmpK36, WP_494812837.1; CirA, WP_499823737.1; FyuA, WP_000784549.1); for each hit, the native open reading frame was extracted from its true start codon and translated to identify premature stop codons, rather than relying on query-coverage thresholds alone. All-vs-all ANI was calculated with fastANI v1.33 [6]; the resulting distance matrix (d = 100 − ANI) was used to build a neighbour-joining tree and, via average-linkage hierarchical clustering, to define putative clonal groups at ANI thresholds of 99.95%, 99.98% and 99.995%. Porin lesions were mapped onto this phylogeny to assess monophyly. To contextualise isolates against a concurrent Romanian inpatient CRKP collection [7] (ENA: PRJEB105915), core-genome SNP analysis was performed for all 44 ST383 isolates (12 from this study, 32 inpatient) using snippy v4.6.0 (assembly-contig mode) against the complete ST383 chromosome CP141905.1. A core alignment was generated with snippy-core; pairwise core-SNP distances were computed and a < 20-SNP transmission threshold applied, consistent with published hospital-outbreak genomics standards.

Statistical analysis. Fisher's exact test was used for categorical co-occurrence (*armA*/*iuc*); the Mann-Whitney U test was used for MIC comparisons between genotype groups (e.g. NDM-positive versus NDM-negative, OmpK35-truncated versus OmpK35-intact). A two-sided $p < 0.05$ was considered

significant. Given the modest sample size and the multiple, partially overlapping genotype subgroups analysed, these comparisons are reported as descriptive/exploratory rather than confirmatory.

Results

Cohort characteristics. Median patient age was 67 years (IQR 22; range 1–89); 11 (44%) were female and 14 (56%) were male. Isolates were obtained either from clinical infection sites or from rectal screening for CRKP carriage. Ten isolates were recovered from respiratory tract infections/colonisation, 6 from urinary tract infections, 5 from skin/soft tissue infections, 3 from bloodstream infections and 1 from an intra-abdominal infection. CRKP infection was the primary diagnosis in 20/25 (75%) cases; among the remaining five patients it was a secondary finding (urinary tract infection or infected pressure ulcer during hospital stay) or colonisation. All patients had documented prior hospital exposure; four had positive CRKP rectal-carriage screening on surveillance. No patient had a prior history of cefiderocol treatment. Six (24%) patients were managed as outpatients (including three cases of asymptomatic bacteriuria), 8 (32%) in general wards, and 11 (44%) in intensive care. One paediatric case, referred from another hospital, lacked accompanying clinical data.

Antimicrobial susceptibility. All 25 isolates were resistant to most tested agents. Susceptibility was retained in a minority for ceftazidime-avibactam (6/25), imipenem-relebactam (5/25), amikacin (3/25), gentamicin (5/25), colistin (4/25) and fosfomycin (1/25). All 25 isolates were cefiderocol-resistant by EUCAST v14.0 criteria (MIC 4–32 mg/L) and all remained susceptible to aztreonam-avibactam. Four isolates (16%) were MDR; 21 (84%) were XDR, of which 16 were susceptible only to aztreonam-avibactam.

Assembly quality and clonal structure. Median contig number was 11 (range 5–65); median N50 was 5.29 Mb (range 0.21–5.54 Mb). Isolate 92 was a contiguity outlier (65 contigs, N50 0.21 Mb) and is flagged throughout. Kleborate resolved seven sequence types (Table 1; Fig. 3): ST383 (n = 12, 48%) and ST101 (n = 8, 32%) accounted for 80% of isolates; ST11-1LV, ST15, ST35, ST147 and ST258 each occurred once. All-vs-all ANI (Fig. 1) identified two high-similarity blocks corresponding to ST383 and ST101. Average-linkage clustering at ANI ≥ 99.98% defined a six-isolate ST383 group (17, 25, 60, 91, 93, 94), a five-isolate ST101 group (01, 09, 23, 79, 81), and a pair (03, 42); within the ST383 group, one pair (60/91) reached ANI ≥ 99.995%.

Resistome and plasmidome. Acquired β-lactamase genes and key plasmid replicons are shown in Fig. 2 and summarised in Table 1. NDM-5 was the most frequent carbapenemase (n = 10, all ST383), followed by NDM-1 (n = 6, across ST101/ST15/ST147/ST11-1LV) and OXA-48 (n = 11, in ST101, ST15, ST383). Nine isolates co-harboured an NDM variant with OXA-48. Two isolates (26, 89) carried OXA-48 alone. OXA-181 (ST35, isolate 90), OXA-232 (ST383, isolate 60, co-carried with NDM-5), and KPC-2 (ST258 and ST11-1LV, one each) were each detected once. CTX-M-15 was present in 22/25 isolates. PlasmidFinder detected 18 replicon types; the IncL/M(pOXA-48) replicon was present in 9/11 OXA-48-positive isolates across three sequence types, while isolates 88 and 92 (both ST383) carried OXA-48 on a non-IncL/M

backbone. Isolate 60 carried OXA-232 without the IncL/M(pOXA-48) replicon. The IncHI1B+IncFIB(Mar) (pNDM-MAR-like) replicon combination co-occurred with NDM-5 in all ten NDM-5-positive ST383 isolates. IncX3 replicons were restricted to the three sporadic non-ST383/non-ST101 introductions (06, 51, 90).

armA–iuc co-segregation. All 8/8 aerobactin-positive isolates (03, 25, 42, 46, 60, 88, 92, 94; all ST383) carried *armA*; 0/17 aerobactin-negative isolates did so ($p < 0.001$, Fisher's exact test). All eight also carried NDM-5.

Cefiderocol resistance mechanisms. All 25 isolates were CirA-intact (> 99% identity, full length), excluding siderophore-receptor loss as a contributory mechanism in this cohort. Apparent FyuA absence in 13/25 isolates corresponded exactly to absence of the yersiniabactin/ICEKp locus on Kleborate typing rather than an independent loss event. OmpK35 carried a premature stop codon in 16/25 isolates (64%), via four distinct lesions confirmed by translation of the native open reading frame: a frameshift truncating the protein at residue 63, present in all eight ST101 isolates (01, 09, 23, 26, 40, 79, 81, 89); a second, unrelated frameshift truncating at residue 89, in ST11-1LV (06) and ST258 (51); a third lesion, consistent with an inserted mobile element after codon 34 (truncating at residue 44), in five ST383 isolates (17, 42, 60, 88, 93); and a fourth, singleton frameshift truncating at residue 91 in the ST147 isolate (14). OmpK36 was intact (native start to natural C-terminus) in all 25 isolates, including isolate 24, in which only the extreme N-terminal ~ 50 residues could not be unambiguously resolved from the assembly.

On the ANI phylogeny, the ST101 OmpK35 lesion (residue 63) was monophyletic: all eight carriers formed a single clade with no porin-intact isolate nested within it. The ST383 lesion (residue 44) was not monophyletic: its five carriers were interspersed with porin-intact ST383 isolates at multiple points in the tree.

Isolates carrying only OXA-48 or OXA-181 had the lowest cefiderocol MICs (4 mg/L); KPC-only/KPC-combination strains also showed MIC 4 mg/L. NDM-carrying isolates showed higher MICs (mean 15.8 vs 9.1 mg/L in NDM-negative isolates). OmpK35-truncated isolates showed a similar directional trend versus porin-intact isolates (mean 15.75 vs 10.67 mg/L; median 16 vs 8), not reaching significance at this sample size (Mann-Whitney U, $p = 0.24$). Two carbapenemase-negative isolates (79, 81) showed cefiderocol MICs of 16 mg/L, attributable to dual OmpK35/OmpK36 porin truncation combined with CTX-M-15. Isolates sharing an identical genotype (carbapenemase class and OmpK35 status) did not share a uniform MIC: the seven NDM + OXA-48/OmpK35-truncated isolates ranged 8–32 mg/L, and the seven NDM + OXA-48/OmpK35-intact isolates ranged 4–32 mg/L. Isolates 42 and 93 were genotypically indistinguishable at every locus screened (ST383, NDM-5, OXA-48, CTX-M-15-positive, identical OmpK35 truncation) yet differed four-fold in MIC (32 vs 8 mg/L).

Convergence of carbapenem resistance and hypervirulence. Eight ST383 isolates (03, 25, 42, 46, 60, 88, 92, 94) carried the aerobactin biosynthesis locus (*iucABCD*/*iutA*) and accordingly received a Kleborate virulence score of 3 (Fig. 3); no isolate carried *rmpA* or *rmpA2* (hypermucoviscosity regulators) or the colibactin locus. All eight aerobactin-positive isolates carried NDM-5, and six of the eight additionally

carried OXA-48, meeting operational criteria for convergent CR-hvKp. The tight co-segregation of *armA* with *iuc* (0/17 aerobactin-negative isolates carried *armA*) further indicates that pan-aminoglycoside resistance is physically linked to, rather than independently acquired alongside, the virulence plasmid in this lineage. Yersiniabactin (*ybt 9*/ICEKp3) was present in all eight ST101 isolates and in several sporadic STs, consistent with its frequent carriage on the ICEKp3 mobile element; ICEKp10, the colibactin-associated element, was not detected in this cohort.

Immune-chromatographic testing accuracy. RESIST-3 O.K.N. results, which resolve OXA-48-, KPC- and NDM-type carbapenemases on three separate lines, were discordant with WGS in 4/25 (16%) isolates, comprising false-positive phenotypic results and one mechanistic misattribution. Three isolates (09, 86, 91) tested carbapenemase-positive phenotypically without a corresponding WGS-detected gene. Isolate 09 (ST101) tested NDM-positive but carries only the ST101 OmpK35/OmpK36 truncation and no carbapenemase gene; its cefiderocol MIC was 16 mg/L (consistent with porin-mediated resistance) and its CZA MIC was ≤ 4 mg/L (susceptible), arguing against functional NDM production. Isolate 86 (ST383, unusual K-type KL104, divergent from the K30 typical of ST383) tested NDM-positive but carries no carbapenemase gene by WGS, and may represent a distinct carbapenemase-negative ST383 sub-lineage. Isolate 91 (ST383, K30) tested NDM + OXA-48-positive but no carbapenemase was detected by WGS despite ANI clustering at 99.99% with isolate 93 (NDM-5 + OXA-48-positive); its cefiderocol MIC (32 mg/L, the cohort's highest) is circumstantially consistent with cryptic carbapenemase activity below assembly-detection threshold, possibly reflecting low-copy-number plasmid carriage or culture-associated plasmid loss. A fourth isolate (90, ST35) was reported KPC-positive phenotypically but WGS revealed OXA-181, an OXA-48-subfamily variant not inhibited by relebactam; concordantly, isolate 90 showed CZA MIC ≤ 4 mg/L (susceptible, consistent with avibactam-inhibited OXA-181) and imipenem/relebactam MIC ≤ 1 mg/L, a distinction with direct consequences for β-lactam/β-lactamase-inhibitor selection.

Carbapenemase-negative CRKP. Isolates 79 and 81 (ST101) showed carbapenem-resistant phenotypes (meropenem MIC > 32 mg/L) without an acquired carbapenemase gene, carrying the OmpK36 p.134_135insGlyAsp and OmpK35 p.Ile61fs truncations plus CTX-M-15. This truncation is shared by descent across all eight ST101 isolates; 79 and 81 are the two members lacking a co-occurring carbapenemase gene. Isolate 79 showed imipenem/relebactam MIC > 2 mg/L and CZA MIC > 8 mg/L (resistant to both), while isolate 81 remained susceptible to both (MIC ≤ 1 and ≤ 4 mg/L respectively) despite identical porin mutations.

Core-SNP analysis with the concurrent inpatient collection. Core-SNP analysis of 44 ST383 isolates (12 this study, 32 inpatient [7]) against CP141905.1 spanned 981 core positions (Fig. 4; Table 2). Pairwise core-SNP distances were median 74 (range 5–396) within our cohort, median 54 (range 0–81) within the inpatient collection, and median 65 (range 3–341) across collections. Seven ST383 pairs fell within the < 20-SNP transmission threshold; the closest, isolate 93 ↔ GH_KPO47-2, differed by 3 SNPs, with five of our isolates (60, 86, 91, 93, 94) each within 10 SNPs of GH_KPO47-2. An independent pair, isolate 17 ↔

GH_KPN31, differed by 10 SNPs. Isolate 92 (654 SNPs vs reference, versus 217–335 for other isolates) was an outlier and excluded from transmission analyses.

Discussion

This genomic survey of *K. pneumoniae* from Romanian patients with prior hospital exposure, including outpatients, yields several findings with direct clinical and surveillance relevance.

The cohort is dominated by two parallel clonal expansions, ST383-K30 and ST101-K17, superimposed on sporadic high-risk-lineage introductions. Two distinct plasmid circuits carry the carbapenemase burden: an IncHI1B/IncFIB(Mar) pNDM-MAR-like plasmid effectively confined to, and clonally propagated within, ST383, and an IncL/M(pOXA-48) plasmid observed across three sequence types, indicating horizontal spread. Nine isolates co-carrying an NDM variant and OXA-48 is clinically significant, since dual carbapenemase production typically precludes the ceftazidime/avibactam + aztreonam salvage regimen unless aztreonam resistance is specifically excluded.

Two-thirds of ST383 isolates are aerobactin-positive and meet the convergent CR-hvKp definition, reproducing the ST383 CR-hvKp phenotype described in other Balkan and Middle Eastern collections [3, 4, 8], but detected here in patients across outpatient, general-ward and ICU settings rather than exclusively in acute inpatient care. The concurrent Romanian inpatient CRKP collection [7] independently identified ST383 as the leading clone in a 101-isolate hospital series with the same NDM-5 + OXA-48 signature; core-SNP analysis confirms this as a single shared genomic population at single-nucleotide resolution, with seven cross-study pairs within the transmission threshold, including a 3-SNP near-identical pair. This is consistent with a lineage that bridges hospital and non-acute care rather than representing independent introductions.

The perfect co-segregation of *armA* with the *iuc* virulence plasmid has direct therapeutic consequences: aminoglycosides, frequently proposed as combination partners for hvKp treatment, are pre-emptively co-selected out of the therapeutic arsenal for all aerobactin-positive isolates in this lineage, reinforcing the value of rapid *armA* detection before initiating aminoglycoside-containing regimens.

Phenotypic carbapenemase testing showed a 16% discordance rate with WGS, including a clinically important OXA-181/KPC misattribution; the carbapenemase-negative CRKP isolates (79, 81) further illustrate that carbapenem-resistant phenotypes without a detected carbapenemase gene warrant porin analysis, consistent with prior reports that dual OmpK35/OmpK36 loss combined with an ESBL background is sufficient to drive high-level carbapenem resistance in the absence of any acquired carbapenemase [8, 10].

Regarding cefiderocol, NDM carriage and OmpK35 disruption both trended toward higher MIC, consistent with the ability of metallo-β-lactamases to hydrolyse siderophore-conjugated cephalosporins and with porin loss reducing periplasmic access via the drug's secondary diffusion route. Screening of CirA and FyuA excluded siderophore-receptor loss as a contributory mechanism in this cohort both were

essentially intact or, where apparently absent, tracked a pre-existing yersiniabactin locus deletion rather than a resistance-relevant event; this addresses a mechanism that has been proposed, but not previously excluded by direct screening, in comparable convergent CR-hvKp collections.

The OmpK35 disruption in this dataset is not a single truncating event but four independent premature-stop lesions, distinguishable by their exact truncation point and, for one lesion, by an apparent inserted-sequence signature. Mapping these lesions onto the ANI phylogeny distinguishes two fundamentally different population-genetic processes operating on the same gene within the same collection. The ST101 lesion (truncating at residue 63) is a clonal, lineage-defining feature: all eight ST101 isolates form a single unbroken clade carrying this lesion, with no porin-intact isolate nested inside it, consistent with one truncation event in a common ancestor that was subsequently inherited by the entire clonal expansion sampled here, including isolates recovered from outpatient, ward and ICU settings alike. The ST383 lesion (truncating at residue 44, with a sequence signature consistent with a mobile-element insertion) shows the opposite pattern: its five carriers are phylogenetically interspersed with porin-intact ST383 isolates at multiple points in the tree, rather than forming their own clade, indicating that this lesion arose independently at least twice within the same actively circulating ST383 population. This points to OmpK35 as a recurrent target of loss-of-function mutation under local selective pressure plausibly antibiotic exposure in a population already carrying NDM-5 and receiving repeated β-lactam courses rather than a single imported event, a distinction that would not have been apparent from gene-presence screening alone.

However, genotype did not translate into a deterministic MIC: isolates matched at every screened locus, including carbapenemase gene, variant, ESBL background and OmpK35 status, differed up to four-fold in MIC. Isolates 42 and 93 are indistinguishable across every genomic resistance determinant screened here yet differ four-fold in cefiderocol MIC. Expression-level determinants outside current gene-presence screening approaches for example, regulatory mutations affecting efflux pump expression, or plasmid/gene copy-number variation not resolved from draft assemblies likely also shape the final phenotype. Genotype-based cefiderocol MIC prediction in this lineage should therefore be regarded as directional rather than deterministic, and cannot yet substitute for direct phenotypic testing even when the major resistance genotype is known.

Finally, universal fluoroquinolone resistance was observed: 24/25 isolates carried GyrA+ParC dual QRDR mutations, and the remaining isolate (90) nonetheless showed elevated ciprofloxacin/levofloxacin MICs, suggesting efflux- or porin-mediated accumulation reduction. Fluoroquinolones are therefore effectively unusable as empirical therapy in this population.

Limitations. This analysis is based on Oxford Nanopore long-read assemblies rather than fully closed, polished genomes; assembly contiguity was variable, which may affect resolution of plasmid boundaries. Plasmid replicon co-occurrence was inferred from marker presence rather than circular assembly. Detailed patient-level epidemiological metadata beyond hospitalisation and ward status were not available. The collection derives from a single institution and may not represent *K. pneumoniae*

circulating across Romania more broadly. The residual, genotype-unexplained variance in cefiderocol MIC was not further investigated at the expression or copy-number level here. The putative mobile-element insertion in OmpK35 in five ST383 isolates was characterised by sequence context rather than formal identification against an IS-element reference database.

Conclusions

Whole-genome sequencing of 25 clinical *K. pneumoniae* isolates from Romanian patients with prior hospital exposure identifies a widely circulating, convergent CR-hvKp ST383 lineage with clonal *armA*-mediated aminoglycoside co-resistance, alongside a parallel ST101-K17 expansion carrying a clonally inherited OmpK35 truncation. All 25 isolates were cefiderocol-resistant; NDM carriage and OmpK35 disruption were the principal, but not fully deterministic, genomic correlates of MIC magnitude. Core-SNP analysis establishes this ST383 lineage as bridging outpatient and inpatient care in Romania, supporting extension of genomic surveillance, including porin and siderophore-receptor screening for cefiderocol resistance, beyond acute inpatient settings.

Declarations

Acknowledgement

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Conflicts of interest.

The authors declare no conflicts of interest.

Data availability. Genome assemblies and analysis outputs are available under BioProject accession PRJNA1495471.

Ethics.

This study was conducted in accordance with the Declaration of Helsinki. Data access was granted under ‘Prof. Dr. Matei Balș’ National Institute of Infectious Diseases, Bucharest, Romania Institutional

Review Board approval (registered C9720/19.09.2024). All data were fully anonymised prior to processing; individual written informed consent was accordingly not required under national legislation and institutional requirements.

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Tables

Tables are available in the Supplementary Files section.

Figures

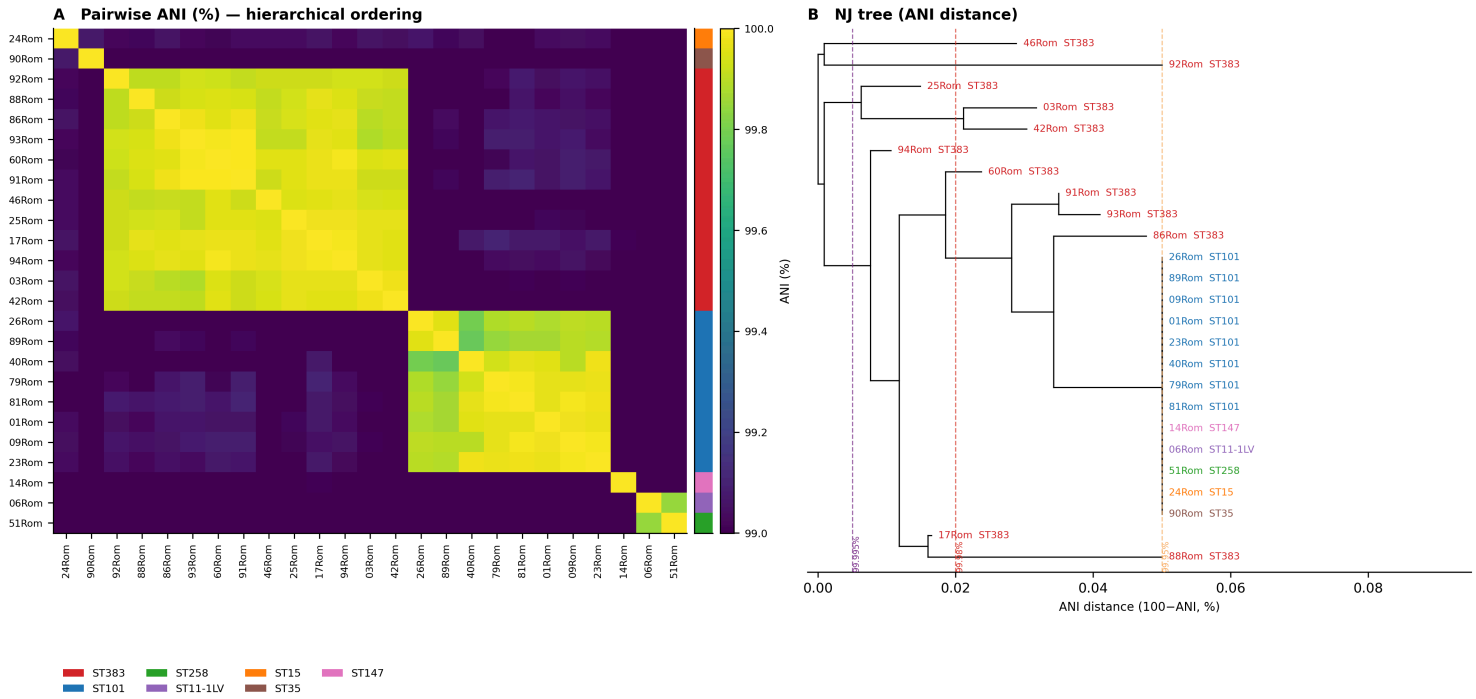


Figure 1

Clonal structure and genomic relatedness of the 25 *K. pneumoniae*isolates. (A) Pairwise all-vs-all average nucleotide identity (ANI, %) computed with fastANI v1.33, displayed after average-linkage hierarchical reordering. The right-hand ribbon encodes sequence type by colour (key shared with panel B). Two high-ANI blocks correspond to the ST383 (n=12) and ST101 (n=8) clonal expansions; isolates belonging to sporadic STs cluster on long branches. Dashed vertical lines in panel B mark ANI thresholds of 99.95%, 99.98% and 99.995% used to define putative transmission groups. (B) Neighbour-joining tree constructed from the ANI distance matrix (d = 100 − ANI). Tip labels are coloured by sequence type. At ANI ≥99.98%, three high-similarity groups are resolved: a six-isolate ST383 cluster (17, 25, 60, 91, 93, 94), a five-isolate ST101 cluster (01, 09, 23, 79, 81), and a two-isolate ST383 pair (03, 42). A single pair (60/91) exceeds ANI 99.995%, consistent with very recent shared ancestry.

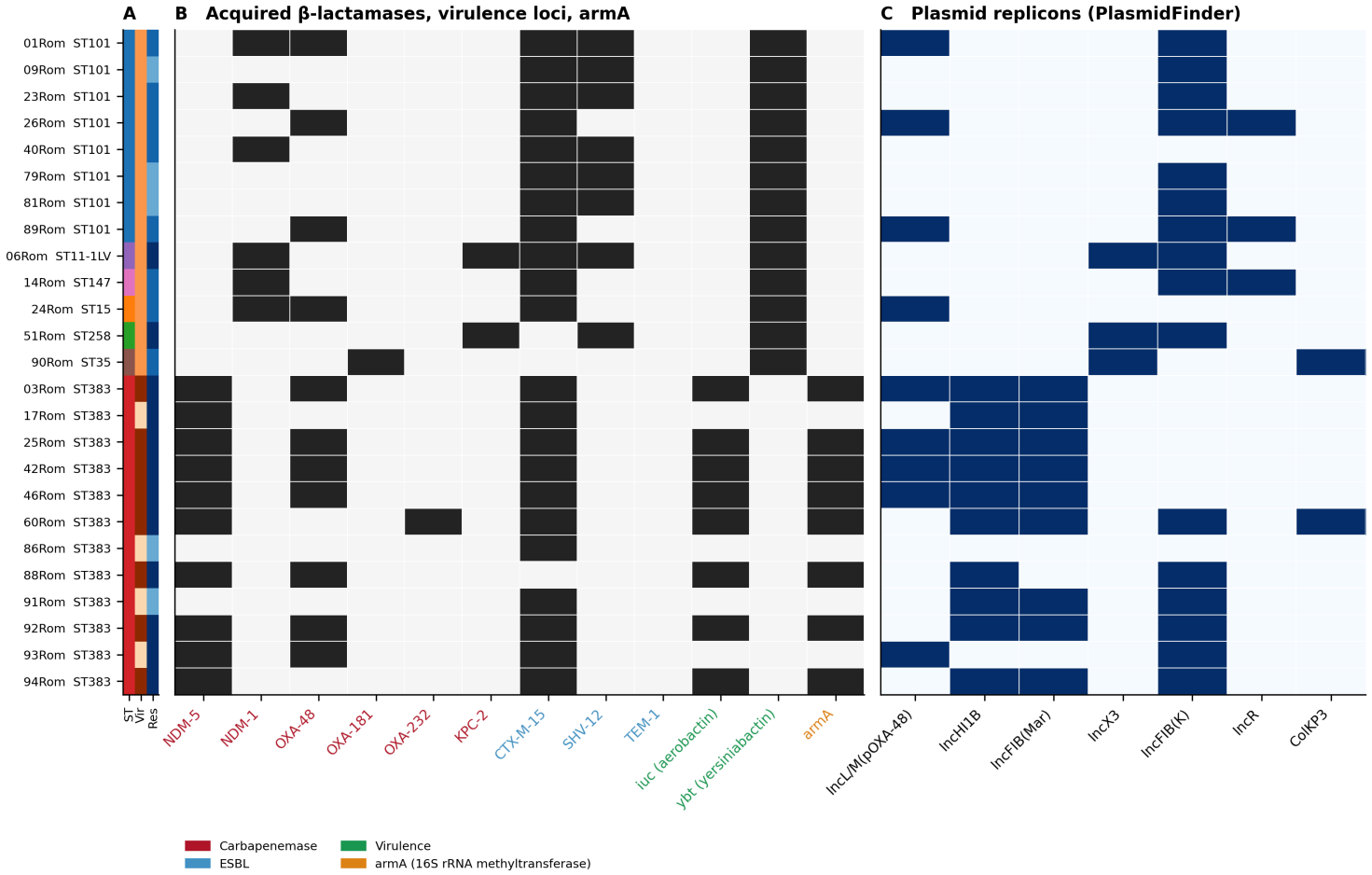


Figure 2

Resistome, virulence loci, *armA* status and plasmid content of the 25 isolates. Rows are isolates sorted by sequence type then laboratory number. (A) Sequence type (ST colour strip), Kleborate virulence score (Vir, orange gradient) and Kleborate resistance score (Res, blue gradient). (B) Presence (filled) or absence (empty) of acquired carbapenemase genes (red labels), ESBL genes (blue labels), virulence-associated mobile loci *iuc* aerobactin and *ybt*yersiniabactin (green labels) and the *armA* 16S rRNA methyltransferase (orange label). The *armA* gene perfectly co-segregates with the *iuc*locus: all 8/8 aerobactin-positive ST383 isolates carry *armA*; 0/17 aerobactin-negative isolates do so. (C) Plasmid replicons detected by PlasmidFinder. The IncHI1B + IncFIB(Mar) co-signature is restricted to ST383 NDM-5 carriers, consistent with a pNDM-MAR-like backbone; the IncL/M(pOXA-48) replicon is shared across four sequence types, indicating inter-clonal horizontal transfer of *bla*OXA-48; the IncX3 replicon is confined to sporadic introductions (06, 51, 90).

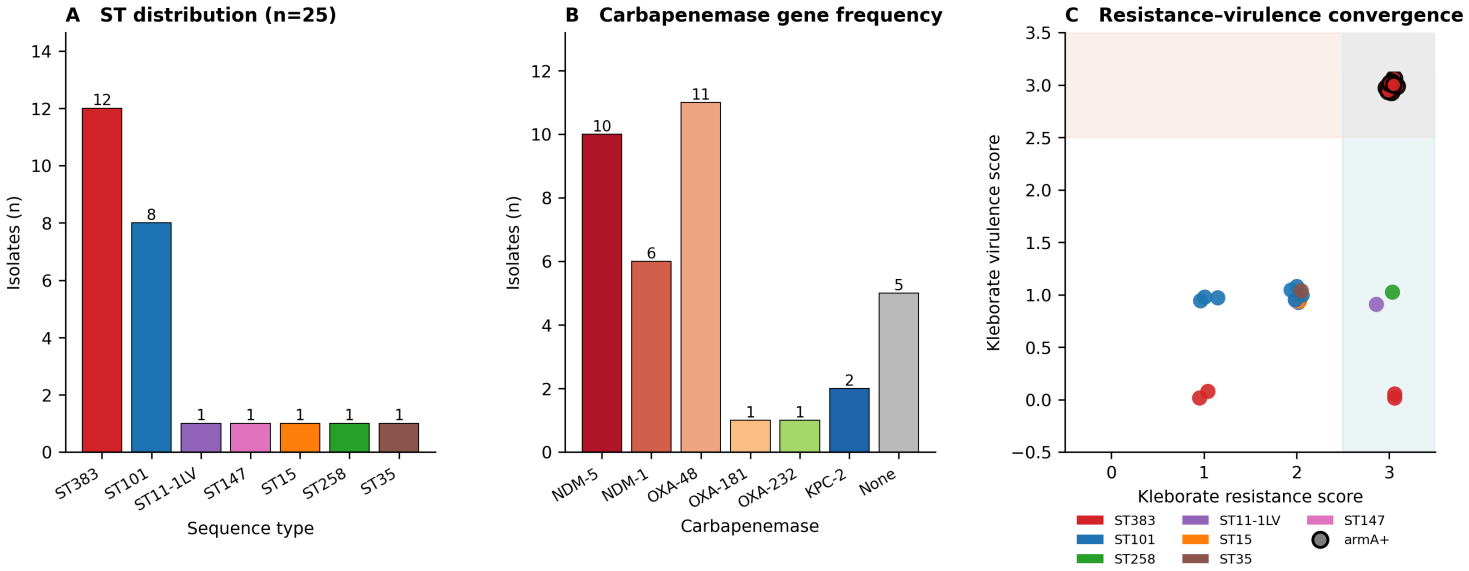


Figure 3

Sequence type distribution, carbapenemase gene frequency and resistance–virulence convergence among the 25 isolates. (A) Sequence-type distribution; bar labels give isolate counts. ST383 (n=12, 48%) and ST101 (n=8, 32%) together account for 80% of the collection. (B) Frequency of individual carbapenemase genes. NDM-5 (n=10) and OXA-48 (n=10) predominate; ten isolates co-carry both. (C) Kleborate resistance score versus virulence score (points jittered ±0.07 for clarity); colours encode sequence type as in panels A and B. Points with a bold black outline carry *armA*. The shaded upper-right quadrant (Vir ≥3, Res 3) highlights convergent carbapenem-resistant hypervirulent *K. pneumoniae*(CR-hvKp); all eight CR-hvKp isolates are ST383 and all carry *armA*.

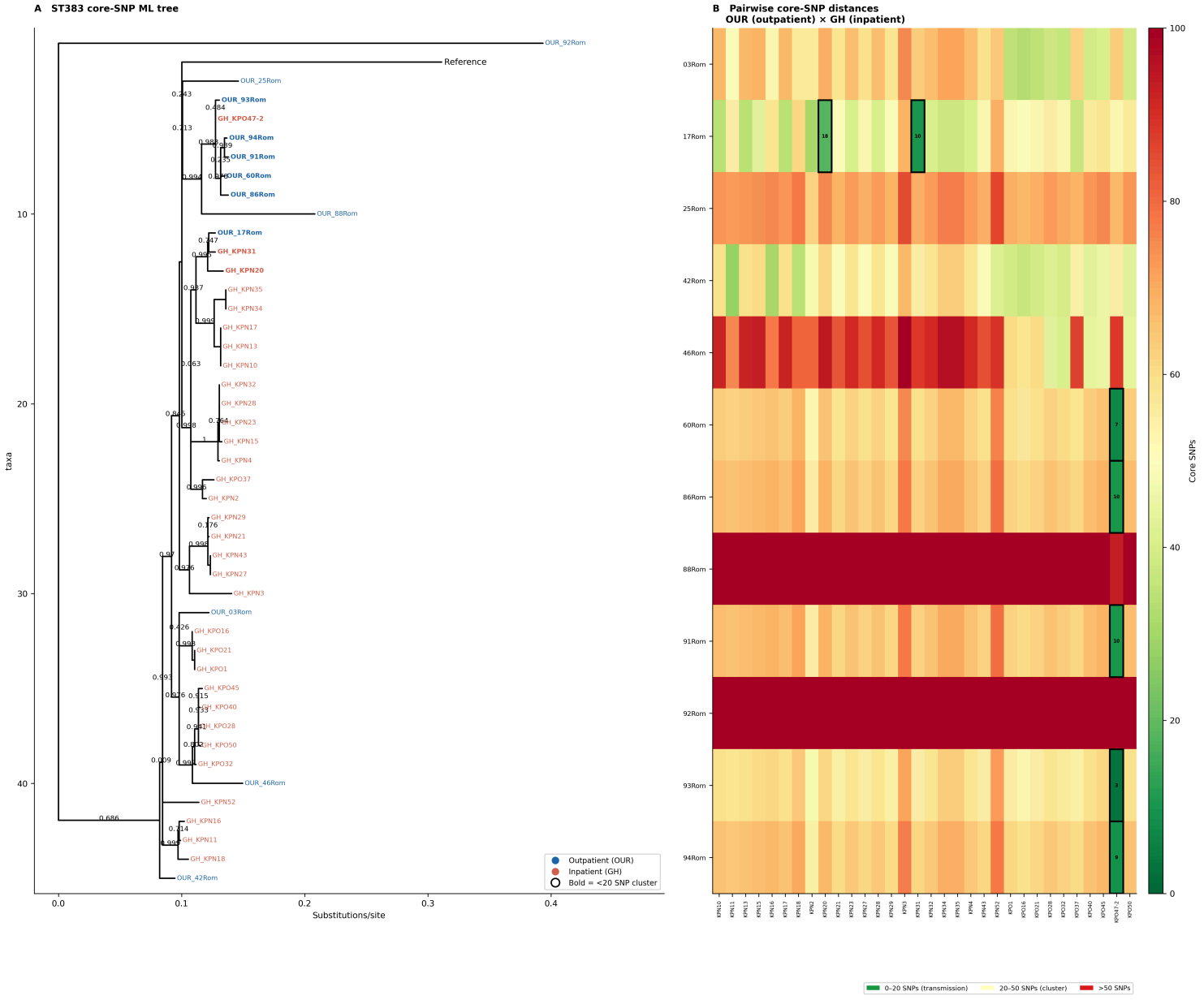


Figure 4

Core-SNP maximum-likelihood phylogeny and pairwise distance heatmap for all 44 ST383 isolates. (A) Maximum-likelihood tree built with FastTree v2 (GTR+CAT model) from 981 parsimony-informative SNP positions in the core alignment generated by snippy v4.6.0 and snippy-core against reference CP141905.1 (complete *K. pneumoniae* ST383 chromosome, NDM-5+OXA-48, 5.32 Mb). Blue circles: outpatient isolates (this study, OUR_, *n=12); red squares: inpatient isolates (Gheorghiță et al. 2026 [7], GH_*, n=32). Bold tip labels indicate isolates involved in cross-study transmission clusters (<20 core SNPs). Tree is midpoint rooted. (B) Pairwise core-SNP distance matrix for outpatient isolates (rows) versus inpatient isolates (columns). Colour scale: green = 0–20 SNPs, yellow = 20–50 SNPs, red >50 SNPs. Black outlines and inset numbers mark the seven outpatient–inpatient pairs within the <20 SNP transmission threshold (see Table 2). GH_KPO47-2 (inpatient) shares a transmission cluster with five outpatient isolates (60, 86, 91, 93, 94) at 3–10 SNPs; isolate 17 forms an independent cross-study

cluster with GH_KPN31 (10 SNPs) and GH_KPN20 (18 SNPs). Multiple inpatient pairs at 0 SNPs (e.g. GH_KPN10/KPN13/KPN17) indicate ongoing nosocomial clonal amplification.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx