CARBAPENEM RESISTANCE SHAPES THE POPULATION STRUCTURE OF KLEBSIELLA PNEUMONIAE

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Introduction

Carbapenems are β-lactam antibiotics that were initially reserved for the treatment of severe multidrug-resistant (MDR) infections, including nosocomial outbreaks of K. pneumoniae. They effectively acylate most known β-lactamases (BLs), including extended-spectrum β-lactamases (ESBLs), which became an issue in the late 1990s. CRKp strains are usually resistant to all β-lactams (including their combinations with BLs inhibitors), and to most other major groups of antibiotics as well. Additional terms in this context have become increasingly used: extended drug resistance (XDR), which defines isolates with acquired non-susceptibility to at least one agent in all but two or fewer antimicrobial categories; and pan-drug resistance (PDR), which defines acquired non-susceptibility to all agents in all antimicrobial categories. Epidemic spread of CRKp, accompanied by multispecies CR outbreaks with increased mortality rates in healthcare associated environment in the 2000s significantly limits treatment options for nosocomial infections caused by Gram-negative pathogens worldwide. In 2017 the World Health Organization included CRKP in a list of priority pathogens for which new antibiotics are urgently needed [1].

The European Surveillance of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) study shows that the degree of resistance correlates with the propensity to spread within the hospital environment and certain lineages of carbapenemase-positive Kp isolates demonstrate the highest transmissibility [2].

Between 2019 and 2023, situation with CR isn't getting better [3] . The incidence of CRKp bloodstream infections (BSI) in Europe increased by 57.5%.

Currently, there are no publicly available statistics on nosocomial infections in Ukraine, but several independent reports suggest an alarming situation. Extremely high levels of carbapenemase producers, including Kp (clones ST147 and ST395) and *Pseudomonas aeruginosa* (clone ST773), have been detected among Ukrainian patients undergoing hospital transfer to Europe and the US [4-7]. Isolation of a PDR hyper-virulent (HV) Kp from Ukrainian war victims has also been reported [8]. The convergence of these two phenotypes, which have the highest clinical relevance and have, until recently, been observed in distinct, non-overlapping parts of the Kp population [9], is another reason for deep concern.

Taxonomy and population structure

The Kp genome is approximately 5–6 Mbp in size, encoding 5,000–6,000 genes, of which about 1,700 are conserved among all members of the species and referred to as the core genome (cg). The remaining set, known as the accessory genome, is represented mostly by unique or rare genes, originating from a wide range of other species, including distantly related. The pangenome (the sum of all core and accessory genes) is extremely diverse and likely exceeds 100,000 protein-coding sequences [10].

Within microbial species, horizontal gene transfer (HGT) and homologous recombination (HR) events promote a more rapid divergence of independently evolving lineages than in eukaryotes. Kp represents apparent confirmation of this concept. As was shown by whole genome sequencing (WGS) data analysis, a substantial proportion of isolates, identified as Kp by routine bacteriological methods, belong to closely related species, forming a group, referred to as *'K.pneumoniae* species complex' (KpSC) with 95–96% average nucleotide identity (ANI) (and ~ 90% ANI with other Klebsiella species) [ibid].

Seven major phylogroups inside KpSC were designated: *K.pneumoniae* subsp. *pneumoniae* (Kp1), that is, *K. pneumoniae sensu stricto* and include the majority of clinically relevant strains; *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2); *K. variicola* subsp.*variicola* (Kp3); *K. quasipneumoniae* subsp. *similipneumoniae* (Kp4); *K. variicola* subsp. *tropica* (Kp5); *K. quasivariicola* (Kp6); *K. africana* (Kp7). Two Klebsiella subspecies (subsp. *rhinoscleromatis,* which causes a progressive and chronic granulomatous infection, and subsp. *ozaenae,* which causes atrophic rhinitis) actually represent the clonal groups (CGs) of Kp (CG3 and CG90) [11].

For epidemiological surveillance purposes, different WGS-based schemes have been proposed. Multilocus Sequence Typing (MLST) of seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*) is the most successful, portable and widely used variant. More advanced schemes have been developed, such as a dual barcoding system based on the 629-loci core genome MLST (scgMLST) that combines multilevel single linkage (MLSL) clustering and life identification numbers (LINs) [12]. It is implemented in a taxonomy identification tool that is publicly accessible through a community-curated platform hosted by the Pasteur Institute (https://bigsdb.pasteur.fr/klebsiella), which enables the upload and identification of genomic sequences by external users. The website also provides access to genomic data and genotypic definitions for isolates of KpSC based on seven-gene MLST, core genome MLST (cgMLST), ribosomal MLST (rMLST), capsular typing (wzc and wzi sequencing), and MLST of virulence gene clusters.

Genomes differing by more than 190 cgMLST loci generally correspond to distinct phylogenetic lineages and are assigned as discrete sublineages (SLs). For clonal groups (CGs), which are usually referred to as 'clones' or 'lineages' and labeled according to the commonly used 7-gene MLST scheme, the threshold was defined as 43 allelic mismatches. An inheritance algorithm allowing the mapping of 7-gene MLST identifiers onto SL and CG partitions has been developed and built in. Using this tool to analyze a dataset of over 7,000 Kp genome sequences demonstrates that more than 90% of clinically relevant

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strains belong to the Kp1 phylogroup, forming 705 SLs and 1,147 CGs. [12].

Although MLST alone could be used to clone's identification, it has limited ability to distinguish CGs whose recent ancestry is affected by chromosomal recombination [10]. And this is an issue, considering the abundant evidence of between-clone horizontal gene transfer (HGT) and the proven fact that large-scale recombination events and capsule switches are major drivers of variation within at least some epidemiologically successful clones [13]. For instance, the CR strain described in Poland and identified by 7-gene MLST as ST23 (a classic HV clone that causes liver abscesses with metastatic spread in otherwise healthy hosts) [14], was mentioned in a 2021 ECDC report as a case of 'hypervirulent Klebsiella pneumoniae ST23 carrying carbapenemase genes' [15]. However, as was noted [16] the strain has atypical for ST23 polysaccharide loci (KL57 and O2v2 instead of usual for ST23 KL1 and O1v2), and currently there is no evidence to suggest that it can cause severe CA infections [17].

The global Kp population likely comprises thousands of distinct phylogenetic lineages or clones. MDR and HV isolates are usually overrepresented in clinical strain collections. As has been shown, a significant portion of the global Kp1 dataset consists of multiple closely related genomes within certain clonal groups (CGs). These genomes contribute disproportionately to the global disease burden and are commonly referred to as 'high-risk' (HiR) or 'global problem' clones. In reduced form (based on a relatively small dataset), the whole-genome phylogeny and schematic Kp population structure are represented in Fig. 1 **(**reproduced from Ref. 10).

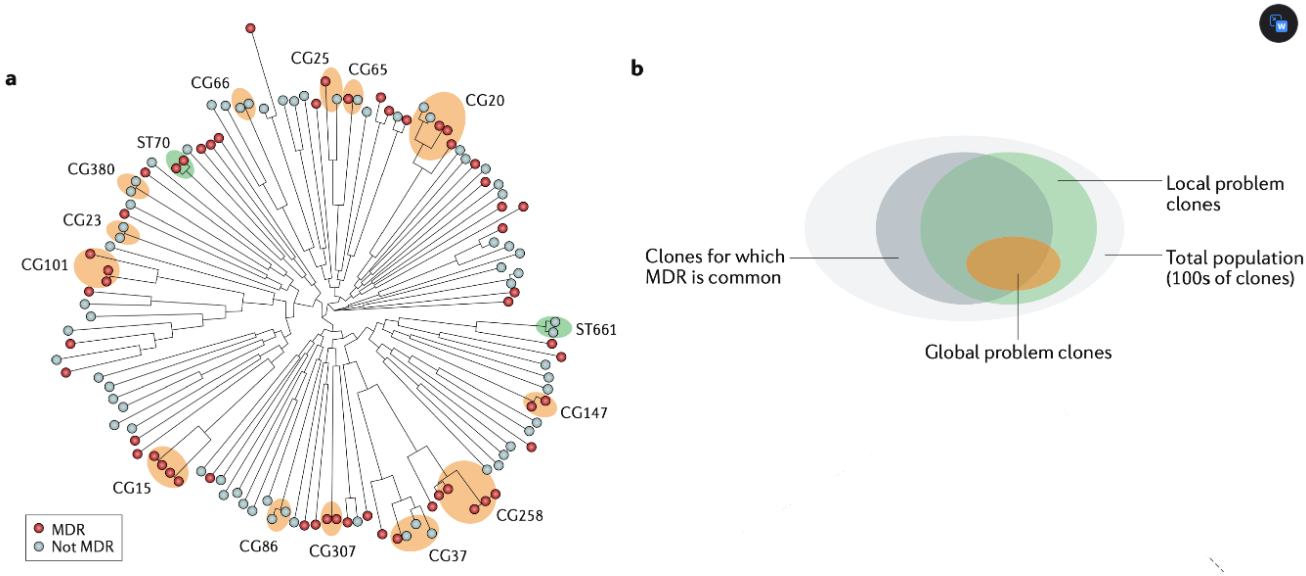

Fig.1 | Klebsiella pneumoniae population structure and global problem clones.
(a) The maximum-likelihood tree was inferred from a core-genome alignment constructed from 83 genomes randomly selected from a global diversity study and 41 genomes representing problem clones;
(b) schematic representation Kp population (distribution map).

Selective pressure definitely facilitates the acquisition of transient MDR phenotypes, which have evolved many times in hundreds of distinct Kp lineages, though this has had limited or no effect on their further dissemination. The loss of acquired mobile genetic elements (MGE) is a natural and predictable process, considering the fitness cost usually associated with their maintenance. Much less explainable are the factors contributing to the epidemiological success of certain clones and their ability to accumulate and maintain a large number of plasmids. Some of these, as was shown, have been maintained throughout decades of clonal expansion, diversification, and widespread dissemination [10]. For example, Kp ST258 has carried the FiBK blaKPC plasmid pKpQiL47 since its emergence in the mid-1990s [18].

Irregularities in the distribution of the accessory genome within the Kp population also remain unexplained, such as the bimodal distribution of resistance-associated MGEs observed between CGs and even within some of them (particularly in CG20 and CG37), where most isolates either carry multiple MGEs (up to 10) or none at all [10]. The commonly acknowledged divergence of resistance and virulent traits, especially noticeable in global problem clones (Fig. 2, image reproduced from ref. [10]), could be a part of this phenomenon.

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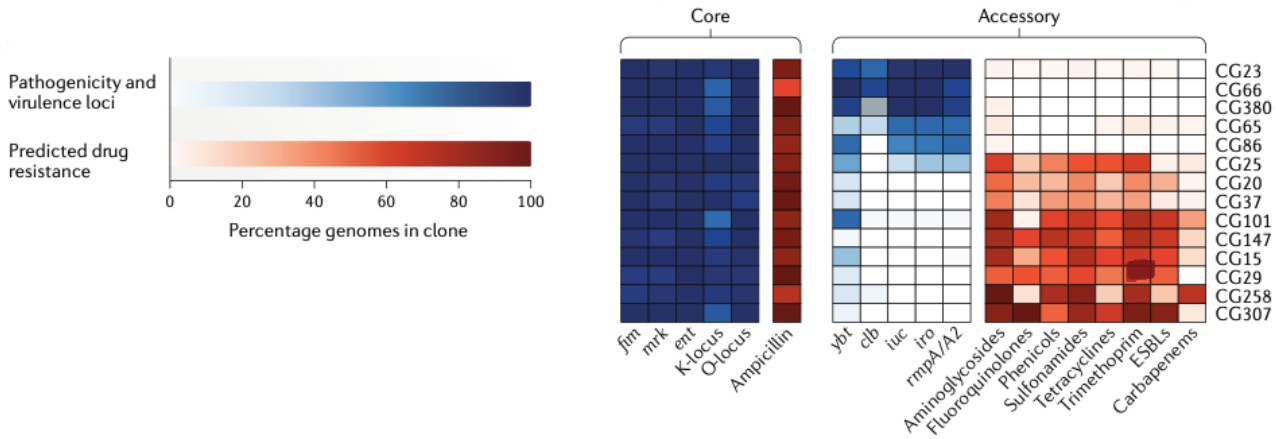

Fig. 2. Distribution of virulence and resistance determinants in global problem clones of Kp

Distribution based on published data of geographically and temporally diverse set of 1,092 genomes from 28 clones (n ≥10 genomes per clone) and n = 95 CG307; clb, colibactin locus; iro, salmochelin locus; iuc, aerobactin biosynthesis locus; rmpA/A2, regulators of mucoid phenotype genes; ST, sequence type; ybt, yersiniabactin locus; ESBLs - extended spectrum β-lactams (e.i. oxyiminocephalosporins or the 'expanded-spectrum cephalosporins)).

Although the convergence of resistance and virulence phenotypes has often been reported (including the appearance of HV in some isolates of CRKpST11 [19, 20] and CR in some isolates of HVKpST23 (as mentioned above ST23KL57 [14]), it has not been a common phenomenon so far [17].

Antibiotic resistance

Kp carries the chromosomally encoded class A β-lactamase HSV (core gene bla_{SHV}), which makes it intrinsically resistant to ampicillin. Notably, this gene was repeatedly captured from the Kp chromosome by the transposase IS26, and HSV became one of the first described mobile β-lactamases [21]. The acquisition of certain point mutations allows some MGE-associated variants of HSV to hydrolyze oxyiminocephalosporins (the 'expanded-spectrum cephalosporins' introduced in the early 1980s), resulting in the ESBL phenotype. Since then, numerous mobile homologs of the bla_{SHV} gene have been widely distributed among different species, present in a wide range of plasmids, and in Kp can co-occur along with their chromosomal ancestor.

Mobilized forms of another Kp core loci fosA (glutathione S-transferase) and oqxAB (efflux pump), being expressed at higher levels than their chromosomal counterparts, can confer clinically relevant resistance to fosfomycin and quinolones, respectively [10].

Certain core genome mutations affecting the functioning of outer membrane porins or efflux pumps (oqxAB and acrAB) can result in resistance to fluoroquinolones, nitrofurantoin, tigecycline, chloramphenicol, colistin, and carbapenems. For example, a specific mutation in OmpK36, combined with the loss of OmpK35, enables Kp isolates bearing the MGE-associated class D β-lactamase OXA-48 to gain clinically relevant levels of carbapenem resistance [10].

Nevertheless, the predominant cause of resistance in Kp is the acquisition of accessory AMR genes mainly via large conjugative plasmids, rather than chromosomal mutations alone. For example, in a single ST258 Kp isolate, four plasmids containing 24 different resistance genes were identified [22]. The presence on plasmids of other antibiotic resistance determinants, as well as other genetic elements that mitigate the fitness cost or confer other advantages, provides an easy mechanism for the spread of carbapenemase genes even in the absence of carbapenem selection. Over 400 acquired AMR genes are present in the Kp genomes, which is twice the number found in E. coli [23].

Since the late 90s, the most influential group, in terms of impact on Kp population structure, has been β-lactamases (BLs) (ESBLs and carbapenemases, in particular). Probably just because β-lactams remain the most prescribed class of antibacterial agents in clinical settings, and carbapenems are still the last-resort antibiotics in the treatment of severe nosocomial infections caused by Gram-negative pathogens.

β-lactamases

The first variants of β-lactamases (BLs) were described in the early 1970s, and since then, their number has been growing rapidly. At the 'Beta-Lactamase Database' (www.bldb.eu [24]), which compiles available information on all known β-lactamases, the total number had reached 8,273 by July 2024. According to the Ambler classification, BLs are divided into four distinct classes, termed A, B, C, and D. Based on active site structure, classes A, C, and D fall into the category of 'serine β-lactamases' (SBLs). Class B comprises a heterogeneous group of zinc metalloenzymes, or 'metallo-β-lactamases' (MBLs) [25]. Within each class, a few enzyme families have been more successful and widely disseminated than others. Most of them belong to Class A [ibid]:

- TEM (the first plasmid-borne BL identified in Gram-negative bacteria);
- SHV (an allelic variant of the chromosomal β-lactamase gene of K. pneumoniae);

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- CTX-M (cefotaximase, which is inherently active against oxyiminocephalosporins);
- KPC (K. pneumoniae carbapenemase, an exceptionally successful enzyme that will be discussed later).

New Delhi MBL (NDM) and Verona integron-encoded MBL (VIM) represent Class B MBLs, while CMY and ADC belong to Class C. All key enzyme families in Class D are referred to as oxacillinases (OXA), as they typically hydrolyze oxacillin, methicillin, and cloxacillin more effectively than benzylpenicillin [ibid].

The evolutionary success of BLs is determined by their quick adaptation to new types of substrates and their association with a variety of MGE that mediate effective inter-replicon and cell-to-cell dissemination. The acquisition of certain point mutations enables TEM and SHV to hydrolyze oxyiminocephalosporins (the 'expanded-spectrum cephalosporins' introduced in the early 1980s), resulting in the so-called 'extended-spectrum' phenotype, [ibid]. Soon after the introduction of imipenem in combination with cilastatin, new BLs with carbapenem-hydrolyzing activity emerged: class B MBLs NDM, which hydrolyze penicillins, cephalosporins, and carbapenems (except aztreonam), and class A KPC, which hydrolyzes all the antibiotics listed, including aztreonam. It is worth noting, both (NDM and KPC) was first identified in Kp, as well as carbapenemase OXA-48 (which along with quinolone resistance genes *qnrA* and *qnrB* was mobilized from marine bacteria *Shewanella*) and ESBL gene CTX-M (mobilized from environmental Enterobacteriaceae *Kluyvera*) [23].

KPC-2, one of the most successful variants of KPC enzymes, exhibits a 20,000-fold increase in diacylation rate compared to the common TEM-1 β-lactamase [26]. KPC-3 differs from KPC-2 by one H274Y mutation and has activity similar to that of KPC-2 [24]. The appearance of these two enzymes has facilitated the global spread of the first recognized MDR 'high risk' Kp clone ST258, as well as inspired the development of new BL inhibitors (i.e., avibactam, vaborbactam, and relebactam) with excellent KPC-degrading activity in vitro. However, the commercialization of the combination ceftazidime-avibactam (CAZavi) in 2015–2016 confirms the notorious evolutionary potential of this enzyme family. By mid-2022, 65 KPC variants resistant to CAZavi have been described [27].

This new substrate adaptation comes at a cost. Many of these enzymes have decreased stability or reduced activity against other beta-lactams. However, the latter could be easily compensated for by the simultaneous presence of ESBLs, which are often observed in Kp.

Global problem clones

The mutual benefit relationships between Kp ST258 (one of the first 'global problem' clones) and one of the first carbapenemases, clearly reflect impact of carbapenem resistance on population structure of Kp. Gen *bla*$_{KPC}$ in a non-ST258 Kp was first identified in 1996 in the US, but large outbreaks due to this strain were not reported [28]. At the beginning of 2000, several hospital outbreaks with CRKp occurred in the USA (nineteen isolates of Kp producing KPC-2 enzyme were recovered from seven hospitals in New York City) [29].

Outside the US, CRKP with *bla*$_{KPC}$ was first isolated in France in 2005 from a patient who had previously been hospitalized in New York. This was followed by reported outbreaks of difficult-to-treat XDR Kp infections in Israel, Greece, Colombia, and China. At the Tel Aviv Sourasky Medical Center, Kp bearing *bla*$_{KPC-3}$ was found to be related to strains isolated in New York [30]. In 2009, these strains were identified by MLST as ST258 [29]. Phylogenetic analysis demonstrates that the ancestor of ST258 emerged from its diverse parental clonal group around 1995 and likely acquired *bla*$_{KPC}$ prior to dissemination [18].

The population of ST258 Kp is represented by two well-defined lineages: clade I (associated with KPC-2) and clade II (associated with KPC-3) [31]. Clade II of ST258 is a 'hybrid' clone created by a large recombination event between ST11 (80% of the genome) and ST442, from which it obtained a 1.1-Mbp area, comprising the integrative conjugative element ICEKp258.2. The ICEKp258.2 contains gene clusters for a type IV pilus (i.e., pilV) and a type III restriction-modification system [32]. Clad I diverged from clade II by acquiring a 215-kb area of capsule polysaccharide biosynthesis region (*cps*) from ST42 [ibid]

In 2010, almost all KPC outbreaks in Europe were caused by this Kp clone [33]. Overall, the outbreaks associated with ST258 CRKP have been reported in more than twenty-five countries from four continents [32].

In 2015 *bla*$_{KPC}$ was found in >100 different KpSTs, but pandemic was still driven primarily by the members of CC 258 (a large group, containing dozens of different STs, representing single-locus variants (SLVs) of ST258 [22].

bla$_{KPC}$ in ST 258 is usually associated with IncF-type plasmids and the transposon Tn*4401*. This means that in addition to clonal expansion and plasmid propagation, *bla*$_{KPC}$ can disseminate through transposon mediated HGT (including transmission between different types of plasmids across multiple genera). In other (non ST258 Kp isolates, or in non Kp species) *bla*$_{KPC}$ has been found in other (non-Tn4401) MGEs, which, nevertheless contain genetic remnants of Tn4401 [31].

The global spread of Kp CG258 has been accompanied by the rapid dissemination of KPC among other clinically relevant species, including *E. coli, Citrobacter sp., Enterobacter sp., Serratia marcescens, Proteus mirabilis,* and *Morganella morganii,* as well as in non-fermentative Gram-negative bacilli. According to the Annual ECDC Report published in 2022, KPC was detected even more often in *Pseudomonas aeruginosa and Acinetobacter baumannii* than in Kp [34].

A typical outbreak of multispecies, plasmid-borne blaKPC carbapenemase in Enterobacterales (KPC-E) was monitored and well described in the UK [35]. Two large teaching hospitals in Manchester (formerly known as CMFT and UHSM) have experienced KPC-E cases since 2009. Sequencing of archived isolates collected between 2009 and 2011 demonstrates that the earliest CR strains in the collection were KPC-producing Kp ST258 and ST11 in both hospital settings, followed by multiple diverse clones

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and species emerging in 2010 and 2011, with blaKPC in at least 30 new species-ST groups per year. Following a blaKPC-2-ST216-Escherichia coli outbreak on two cardiac wards in 2015, intensive infection prevention and control (IPC) measures were implemented (e.g., closure of wards, terminal cleaning with hypochlorite, decontamination with hydrogen peroxide vapor, and plumbing infrastructure replacement). Screening of at-risk patients, along with the separation of CRE-positive individuals in a cohort ward, was conducted after reopening. However, these measures had only a transient effect. Early environmental sampling suggested rapid recolonization of wastewater sites with KPC-E and patient CRE acquisitions, and the first CR species to reappear was K. pneumoniae [36]. The sampling undertaken in 2016–2017, showed a decline in CR E.coli, but similar polyspecies polyclonal colonization. Thirteen KPC-E species and 109 strains were identified, including *Klebsiella* spp. (*n* = 34), *Enterobacter* spp. (*n* = 25), and *E. coli* (*n* = 11) [37].

In addition to CG258 (represented mostly by ST11, ST340, ST512, and probably ST395 [13], related to ST258) other unrelated high-risk clones currently causing MDR and XDR outbreaks worldwide belong to CG15, CG20 (CG17), CG29, CG37, CG147, and CG307.

Situation in Ukraine

Currently, there is no data on the molecular surveillance of Kp in Ukraine; however, an XDR isolate of ST147, bearing the carbapenemase genes $bla_{\text{NDM-1}}$ and bla_{OXA-48}, linked to the transfer of a Ukrainian patient, has been described in Spain [5]. CG147 (which includes ST147, ST273, and ST392) comprises pandrug-resistant and extensively resistant isolates, carries multiple MGEs and diverse resistance genes, including chromosomal $bla_{\text{NDM-5}}$ [38].

WGS of 37 isolates collected from Ukrainian patients between February and September 2022 was performed at Lund University (Sweden) [8]. Four of them were identified by 7-gene MLST as ST23 (HV clone), nine as ST147, seven as ST307, seventeen as ST395 (which is widely reported in Russia), and one as ST512 (belonging to CG258). All isolates possess bla_{OXA-1}, bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{NDM-1}, and bla_{NDM-6} in different combinations, including ST23, which demonstrates a PDR phenotype and virulence in the G. mellonella infection model.

Hospital admission screening of a single Ukrainian patient (with extensive combined injuries and a history of prior treatment in Ukraine) in Germany revealed eight XDR pathogens from six different species, including Acinetobacter baumannii (ST78, ST2), Klebsiella pneumoniae (n = ST395), Pseudomonas aeruginosa (n = 1; ST1047), Escherichia coli (n = 1; ST46), and Enterobacter cloacae complex (n=1; ST231), as well as six different acquired carbapenemase genes (bla_{IMP-1}, bla_{NDM-1}, bla_{OXA-48}, bla_{NDM-5}, bla_{OXA-72}, bla_{OXA-23}). Both Kp isolates shared a 7-gene MLST sequence type and resistance genes on an IncR plasmid ($bla_{CTX-M-15}$, bla_{OXA-1}, *and catB3*) but differed in cgMLST (by 126 out of 2358 alleles) and carbapenemases (OXA-48 or NDM-1).

All this indicates a somewhat worrying situation within the Ukrainian healthcare system regarding CR nosocomial infections and CRKP in particular. Advanced epidemiological monitoring and the implementation of intensive prevention and containment measures are urgently required.

Carbapenem resistance shapes the population structure of *Klebsiella pneumoniae*

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Klebsiella pneumoniae (Kp) is an ubiquitous and highly versatile species from the family Enterobacteriaceae. Remarkable genetic and phenotypic plasticity determines its success in adapting to a wide range of environmental and host-associated niches (e.g., fresh and salt waterwater, soil, plants, insects, birds, and animals, including humans, where it usually persists as a gut commensal, but also can colonize other mucosal surfaces and skin). Certain lineages can cause severe invasive community-acquired (CA) infections, but the majority behave as a typical opportunistic pathogen, persisting asymptomatically till the moment of the host vulnerability. One of the most concerning aspects of Kp's recent evolution is its adaptation to the environment of healthcare facilities. The species is one of the major nosocomial pathogens, and the most frequent cause of neonatal sepsis worldwide. Distinguishing clinical feature of Kp infections is propensity for the development of bacteremia and metastatic spread to unusual and often multiple locations (e.g., hepatic, and non-hepatic abscesses, meningitis, endophthalmitis, necrotizing fasciitis, etc.). Despite such a prominent clinical relevance per se, Kp has been recognized as a global healthcare threat primarily due to its contribution to the current crisis of carbapenem resistance (CR). Certain epidemiologically successful lineages turned out to be incredibly effective in the accumulation, maintenance, and spread of resistance-associated mobile genetic elements (rMGEs) among other clinically relevant Gram-negative organisms. The aggregation of numerous resistance genes (including those, encoding ESBL's and/or carbapenemases) within a specific Kp genetic background has led to the emergence of global hospital outbreak CRKp clones, followed by multispecies outbreaks of difficult-to-treat CR healthcare associated infections (HAI) worldwide. Recent surveillance efforts and molecular epidemiology studies demonstrate a complex population structure of Kp, characterized by hundreds of deeply branching phylogenetic lineages. These lineages exhibit significant differences in the distribution of genes conferring antibiotic resistance and virulence, as well as variable transmissibility within the hospital environment. This review focuses on understanding the evolution of clinically relevant genetic variants of CRKp, which is crucial for the development of effective control strategies.

Keywords: *Klebsiella pneumoniae*, Carbapenem resistance, population

References

1. World Health Organization. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. World Health Organisation; 2017.

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2. 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. doi:10.1038/s41564-019-0492-8
3. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2023. Stockholm: ECDC; 2024.
4. Fuchs F, Xanthopoulou K, Burgwinkel T, et al. Coexistence of seven different carbapenemase producers in a single hospital admission screening confirmed by whole-genome sequencing. *J Glob Antimicrob Resist*. Published online October 15, 2024. doi:10.1016/j.jgar.2024.09.005
5. Cabello M, Hernández-García M, Maruri-Aransolo A, et al. Occurrence of multi-carbapenemase-producing Enterobacterales in a tertiary hospital in Madrid (Spain): A new epidemiologic scenario. *J Glob Antimicrob Resist*. 2024;38:281-291. doi:10.1016/j.jgar.2024.06.012
6. Hernández-García M, González de Aledo M, Ponce-Alonso M, et al. Simultaneous clonal spread of NDM-1-producing *Pseudomonas aeruginosa* ST773 from Ukrainian patients in the Netherlands and Spain. *IJID Reg*. 2024;12:100415. Published 2024 Jul 30. doi:10.1016/j.ijregi.2024.100415
7. Schultze T, Hogardt M, Velázquez ES, et al. Molecular surveillance of multidrug-resistant Gram-negative bacteria in Ukrainian patients, Germany, March to June 2022. *Euro Surveill*. 2023;28(1):2200850. doi:10.2807/1560-7917.ES.2023.28.1.2200850
8. Ljungquist O, Magda M, Giske CG, et al. Pandrug-resistant Klebsiella pneumoniae isolated from Ukrainian war victims are hypervirulent. *J Infect*. Published online October 11, 2024. doi:10.1016/j.jinf.2024.106312.
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. Published 2019 Apr 15. doi:10.1371/journal.pgen.1008114
10. Wyres KL, Lam MMC, Holt KE. Population genomics of Klebsiella pneumoniae. *Nat Rev Microbiol*. 2020;18(6):344-359. doi:10.1038/s41579-019-0315-1
11. Brisse S, Fevre C, Passet V, et al. Virulent clones of Klebsiella pneumoniae: identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS One*. 2009;4(3):e4982. doi:10.1371/journal.pone.0004982.
12. Hennart M, Guglielmini J, Bridel S, et al. A Dual Barcoding Approach to Bacterial Strain Nomenclature: Genomic Taxonomy of Klebsiella pneumoniae Strains. *Mol Biol Evol*. 2022;39(7):msac135. doi:10.1093/molbev/msac135
13. Wyres KL, Gorrie C, Edwards DJ, et al. Extensive Capsule Locus Variation and Large-Scale Genomic Recombination within the Klebsiella pneumoniae Clonal Group 258. *Genome Biol Evol*. 2015;7(5):1267-1279. Published 2015 Apr 10. doi:10.1093/gbe/evv062
14. Biedrzycka M, Izdebski R, Urbanowicz P et al. MDR carbapenemase-producing Klebsiella pneumoniae of the hypervirulence-associated ST23 clone in Poland, 2009-19. *J Antimicrob Chemother*. 2023;78(4):1132-1134. doi:10.1093/jac/dkad028
15. European Centre for Disease Prevention and Control. Emergence of hypervirulent Klebsiella pneumoniae ST23 carrying carbapenemase genes in EU/EEA countries. 17 March 2021. ECDC: Stockholm; 2021.
16. Lam MMC, Holt KE, Wyres KL. Comment on: MDR carbapenemase-producing Klebsiella pneumoniae of the hypervirulence-associated ST23 clone in Poland, 2009-19. *J Antimicrob Chemother*. 2023;78(4):1132-1134. doi:10.1093/jac/dkad028
17. Lam MMC, Wyres KL, Duchêne S, et al. Population genomics of hypervirulent Klebsiella pneumoniae clonal-group 23 reveals early emergence and rapid global dissemination. *Nat Commun*. 2018;9(1):2703. Published 2018 Jul 13. doi:10.1038/s41467-018-05114-7
18. Bowers JR, Kitchel B, Driebe EM, et al. Genomic Analysis of the Emergence and Rapid Global Dissemination of the Clonal Group 258 Klebsiella pneumoniae Pandemic. *PLoS One*. 2015;10(7):e0133727. Published 2015 Jul 21. doi:10.1371/journal.pone.0133727
19. 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. doi:10.1016/S1473-3099(17)30489-9
20. Zhou K, Xue CX, Xu T, et al. A point mutation in recC associated with subclonal replacement of carbapenem-resistant Klebsiella pneumoniae ST11 in China. *Nat Commun*. 2023;14(1):2464. Published 2023 Apr 28. doi:10.1038/s41467-023-38061-z
21. Ford PJ, Avison MB. Evolutionary mapping of the SHV beta-lactamase and evidence for two separate IS26-dependent blaSHV mobilization events from the Klebsiella pneumoniae chromosome. *J Antimicrob Chemother*. 2004;54(1):69-75. doi:10.1093/jac/dkh251
22. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international hi Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin Microbiol Rev*. 2015;28(3):565-591. doi:10.1128/CMR.00116-14
23. Wyres KL, Holt KE. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. *Curr Opin Microbiol*. 2018;45:131-139. doi:10.1016/j.mib.2018.04.004
24. Naas, T., Oueslati, S., Bonnin, R. A., et al. Beta-Lactamase DataBase (BLDB) – Structure and Function. *J. Enzyme Inhib. Med. Chem*. 2017, 32, 917-919.
25. Tooke CL, Hinchliffe P, Bragginton EC, et al. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *J Mol Biol*. 2019;431(18):3472-3500. doi:10.1016/j.jmb.2019.04.002
26. Mehta SC, Furey IM, Pemberton OA, Boragine DM, Chen Y, Palzkill T. KPC-2 β-lactamase enables carbapenem antibiotic resistance through fast deacylation of the covalent intermediate. *J Biol Chem*. 2021;296:100155. doi:10.1074/jbc.RA120.01505
27. Hobson CA, Pierrat G, Tenaillon O, et al. Klebsiella pneumoniae Carbapenemase Variants Resistant to Ceftazidime-Avibactam: an Evolutionary

DOI: 10.5281/zenodo.15011907

Overview. *Antimicrob Agents Chemother*. 2022;66(9):e0044722. doi:10.1128/aac.00447-22
28. Bradford PA, Bratu S, Urban C, Visalli M, et al. Emergence of carbapenem-resistant Klebsiella species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin Infect Dis. 2004 Jul 1;39(1):55-60. doi: 10.1086/421495. Epub 2004 Jun 14. PMID: 15206053.
29. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing Klebsiella pneumoniae isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother*. 2009;53(8):3365-3370. doi:10.1128/AAC.00126-09
30. Navon-Venezia S, Leavitt A, Schwaber MJ, et al. First report on a hyperepidemic clone of KPC-3-producing Klebsiella pneumoniae in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrob Agents Chemother*. 2009;53(2):818-820. doi:10.1128/AAC.00987-08
31. Deleo FR, Chen L, Porcella SF, et al. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 Klebsiella pneumoniae. *Proc Natl Acad Sci U S A*. 2014;111(13):4988-4993. doi:10.1073/pnas.1321364111
32. Chen L, Mathema B, Pitout JD, DeLeo FR, Kreiswirth BN. Epidemic Klebsiella pneumoniae ST258 is a hybrid strain. *mBio*. 2014;5(3):e01355-14. Published 2014 Jun 24. doi:10.1128/mBio.01355-14
33. Grundmann H, Livermore DM, Giske CG et al. Carbapenem-non- susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro surveillance 2010; 15: pii¼19711.
34. WHO Regional Office for Europe/European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2022 – 2020 data. Copenhagen: WHO Regional Office for Europe; 2022
35. Stoesser N, Phan HTT, Seale AC, et al. Genomic Epidemiology of Complex, Multispecies, Plasmid-Borne *bla*$_{KPC}$Carbapenemase in *Enterobacterales* in the United Kingdom from 2009 to 2014. *Antimicrob Agents Chemother*. 2020;64(5):e02244-19. Published 2020 Apr 21. doi:10.1128/AAC.02244-19
36. Decraene V, Phan HTT, George R, et al. A Large, Refractory Nosocomial Outbreak of Klebsiella pneumoniae Carbapenemase-Producing Escherichia coli Demonstrates Carbapenemase Gene Outbreaks Involving Sink Sites Require Novel Approaches to Infection Control. *Antimicrob Agents Chemother*. 2018;62(12):e01689-18. Published 2018 Nov 26. doi:10.1128/AAC.01689-18
37. Stoesser N, George R, Aiken Z, et al. Genomic epidemiology and longitudinal sampling of ward wastewater environments and patients reveals complexity of the transmission dynamics of *bla*$_{KPC}$-carbapenemase-producing Enterobacterales in a hospital setting. *JAC Antimicrob Resist*. 2024;6(5):dlae140. Published 2024 Sep 3. doi:10.1093/jacamr/dlae140
38. Rodrigues C, Desai S, Passet V, Gajjar D, Brisse S. Genomic evolution of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal group 147. *Microb Genom*. 2022;8(1):000737. doi:10.1099/mgen.0.000737

DOI: 10.5281/zenodo.15011907