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Expert Review of Anti-infective Therapy

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ierz20

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To cite this article: Vincenzo Di Pilato, Simona Pollini, Vivi Miriagou, Gian Maria Rossolini & Marco Maria D'Andrea (2024) Carbapenem-resistant *Klebsiella pneumoniae*: the role of plasmids in emergence, dissemination, and evolution of a major clinical challenge, Expert Review of Anti-infective Therapy, 22:1-3, 25-43, DOI: 10.1080/14787210.2024.2305854

To link to this article: https://doi.org/10.1080/14787210.2024.2305854

EXPERT REVIEW OF ANTI-INFECTIVE THERAPY
2024, VOL. 22, NOS. 1–3, 25–43
https://doi.org/10.1080/14787210.2024.2305854

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REVIEW

Carbapenem-resistant *Klebsiella pneumoniae*: the role of plasmids in emergence, dissemination, and evolution of a major clinical challenge

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ABSTRACT

Introduction: *Klebsiella pneumoniae* is a major agent of healthcare-associated infections and a cause of some community-acquired infections, including severe bacteremic infections associated with metastatic abscesses in liver and other organs. Clinical relevance is compounded by its outstanding propensity to evolve antibiotic resistance. In particular, the emergence and dissemination of carbapenem resistance in *K. pneumoniae* has posed a major challenge due to the few residual treatment options, which have only recently been expanded by some new agents. The epidemiological success of carbapenem-resistant *K. pneumoniae* (CR-Kp) is mainly linked with clonal lineages that produce carbapenem-hydrolyzing enzymes (carbapenemases) encoded by plasmids.

Areas covered: Here, we provide an updated overview on the mechanisms underlying the emergence and dissemination of CR-Kp, focusing on the role that plasmids have played in this phenomenon and in the co-evolution of resistance and virulence in *K. pneumoniae*.

Expert opinion: CR-Kp have disseminated on a global scale, representing one of the most important contemporary public health issues. These strains are almost invariably associated with complex multi-drug resistance (MDR) phenotypes, which can also include recently approved antibiotics. The heterogeneity of the molecular bases responsible for these phenotypes poses significant hurdles for therapeutic and diagnostic purposes.

ARTICLE HISTORY
Received 13 November 2023
Accepted 11 January 2024

KEYWORDS
Klebsiella pneumoniae; carbapenem resistance; carbapenemase-encoding plasmids; mobile genetic elements; virulence; high-risk clones

1. Introduction

Klebsiella pneumoniae is a major pathogen in the nosocomial setting, where it can be responsible for a broad repertoire of healthcare-associated infections (HCAIs). In a recent survey on HCAIs in Europe, *K. pneumoniae* ranked third among isolated microorganisms, with a prevalence of 10.4% in acute-care hospitals and of 11.4% in long-term care facilities [1]. Outside Europe, *K. pneumoniae* ranked first in some African countries (with prevalences higher than 20%) [2,3], third in the U.S.A. (with prevalence of 9.9%) [4], and first to third in some Asian countries (with prevalences ranging from 7.3 to 15%) [5,6]. *K. pneumoniae* can also be a cause of community-onset infections, including urinary tract infections, pneumonia, skin and soft tissue infections, and even severe bacteremic infections associated with abscesses in the liver and in other sites, caused by strains with increased virulence (hypervirulent) [7–10].

The clinical relevance of *K. pneumoniae* is compounded by its notable propensity to acquire resistance to all classes of potentially active antibiotics, including carbapenems, which have been the cornerstone for treatment of severe infections by Enterobacterales resistant to expanded-spectrum cephalosporins and fluoroquinolones. Indeed, among Enterobacterales, *K. pneumoniae* is the species most affected by carbapenem resistance, which in some settings has reached remarkably high rates (e. g., 67% in Greece, 65% in Iran, 64% in Russia, 57% in India, 50% in Saudi Arabia, 45% in Peru, 33% in Italy, 27% in China, 26% in Argentina, 24% in Brazil) [6,11], with an outstanding burden at the global level [12,13]. Consequently, carbapenem-resistant *K. pneumoniae* (CR-Kp) is currently considered among major public health challenges, and has been included in the WHO priority list of critical resistant pathogens for discovery and development of new antimicrobials [14].

In CR-Kp, resistance is mostly due to the acquisition of various genes encoding β-lactamases capable of degrading carbapenems (carbapenemases). The most prevalent carbapenemases detected in *K. pneumoniae* include KPC-type (molecular class A, active-site serine), OXA-48-type (molecular class D, active-site serine), and NDM-, VIM-, and IMP-type (molecular class B, zinc metallo-enzymes) [15]. Other carbapenemases have also been rarely reported (e. g., GES-5, SFC-1, NMC-A, BKC-1 and SME-1 of molecular class A, AIM-1, and SIM-1 of molecular class B, and OXA-427 of molecular class D) [16–19], but their contribution to carbapenem resistance in this species has remained marginal so far, and their detection is often restricted to single countries. Porin alterations, leading to

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/14787210.2024.2305854

Article highlights

- *Klebsiella pneumoniae* is a highly versatile pathogen, playing a primary role in opportunistic healthcare-associated infections, and being relevant also in some community-acquired infections.
- *K. pneumoniae* exhibits high propensity to develop resistance to antibiotics, including carbapenems, and carbapenem-resistant *K. pneumoniae* (CR-Kp) contribute for the majority of carbapenem-resistant Enterobacterales globally.
- In CR-Kp, resistance to carbapenems is mostly due to acquisition of plasmids encoding carbapenemases of different types and, often, additional resistance determinants.
- Highly diverse carbapenemase-encoding plasmids have evolved, contributing to successful dissemination of carbapenem resistance among some high-risk clonal lineages of *K. pneumoniae*.
- Recombination events involving plasmids and/or associated mobile genetic elements can contribute to accretion of resistance determinants and expansion of the resistance phenotype.
- Plasticity of plasmids and cognate mobile genetic elements can also modulate resistance via alteration of the gene dosage, affecting resistance to some novel β-lactamase inhibitor combinations.
- Convergence in a single isolate of resistance and virulence plasmids, or of hybrid derivatives thereof, can promote the emergence of carbapenem-resistant and highly virulent pathotypes of *K. pneumoniae*, representing a worrisome clinical evolution.

reduced carbapenems entry into the periplasmic space, can also contribute to carbapenem resistance, and to increase the resistance level when present in combination with carbapenemases, or even extended-spectrum or AmpC-type β-lactamases with weak carbapenemase activity [20,21].

Carbapenemases are typically plasmid-encoded enzymes, so that plasmids have played a very major role in the emergence and dissemination of carbapenem resistance in *K. pneumoniae*, while the association of carbapenemase-encoding plasmids with certain successful clonal lineages provided formidable platforms for the epidemic/pandemic propagation of these resistance vehicles.

The scope of this review article is to provide an updated overview on the contribution of resistance plasmids to the evolution of the ongoing pandemic challenge represented by CR-Kp.

2. Epidemiology, evolutionary trajectories, and clinical challenges of the major plasmid-mediated carbapenemases in *K. pneumoniae*

So far, KPC-type carbapenemases have experienced the broadest distribution of all carbapenem-hydrolyzing enzymes. KPC was first described in the U.S.A. in 1996 [22]. Thenceforth, these resistance determinants, mainly represented by the KPC-2 and KPC-3 variants, have become rapidly endemic in the Americas, southern Europe and some parts of Asia [15]. KPC carbapenemases exhibit a broad spectrum of activity, including penicillins, cephalosporins, aztreonam, and carbapenems, and are not efficiently inhibited by older β-lactam-based β-lactamase inhibitors (i. e., clavulanic acid, tazobactam, sulbactam). Enmetazobactam is a somewhat better inhibitor of KPC enzymes, but yet unable to restore activity of cefepime against most KPC-producing strains, while novel non β-lactam-based β-lactamase inhibitors, such as diazabicyclooctanes (e. g., avibactam and relebactam) and boronates (e. g., vaborbactam), are usually able to efficiently inhibit these enzymes and restore the antimicrobial activity of older β-lactams (e. g., ceftazidime and carbapenems) against KPC-producing strains [23]. In fact, these new β-lactam plus β-lactamase inhibitor combinations (BLICs) have become the standard-of-care for infections caused by CR-Kp producing KPC-type enzymes (KPC-Kp), given their outstanding superiority *vs.* older colistin-based regimens [24,25]. However, KPC-type carbapenemases exhibit a remarkable evolutionary potential, and a number of novel KPC variants have recently emerged. These enzymes differ from their ancestors (i. e., KPC-2 or KPC-3) by single amino acid substitutions (e. g., the D179Y amino acid substitution in the Ω-loop of KPC-3, leading to KPC-31) and/or by small insertions/deletions in certain protein domains (e. g., in the loop 237–243 and in the loop 266–275), which can confer resistance to ceftazidime/avibactam (CZA), the first of the new BLICs introduced in clinical practice [26,27]. Several of these KPC variants also differ by other resistance phenotypes (e. g., lower resistance levels to carbapenems, aztreonam, piperacillin/tazobactam and decreased susceptibility to cefiderocol) [28,29], with relevant consequences in terms of identification of the resistance mechanisms and, possibly, of therapeutic approach [27,30]. The emergence of KPC variants resistant to CZA represents a matter of increasing concern in settings of KPC-Kp endemicity, while the plasmid-encoded nature of these enzymes may facilitate their rapid dissemination in the clinical setting [31,32].

Emergence of the IMP- and VIM-type metallo-β-lactamases (MBLs) in *K. pneumoniae* also dates back to the early 1990s and 2000s, respectively, with initial sporadic reports [33–38]. In the following years, these enzymes experienced dissemination in some geographic areas (e. g., Greece, for VIM-type enzymes, and the Asia-Pacific region for IMP-type enzymes) [39,40], but a broader dissemination at the global level has not been observed. On the other hand, NDM-type MBLs and OXA-48-like enzymes were detected more recently (in the first decade of 2000) [41,42], but have experienced an overall broader dissemination at the global level, becoming the most prevalent carbapenemases in Asia, Middle East, and North Africa and also in some areas of the European continent (e. g., Poland, the Balkan region, and UK) [43,44].

MBLs can confer broad-spectrum β-lactam resistance, including penicillins, cephalosporins, and carbapenems, while monobactams are not hydrolyzed by these enzymes. Notably, MBLs are not inhibited by the conventional β-lactam-based β-lactamase inhibitors nor by the novel inhibitors that have entered clinical practice, including avibactam, relebactam, and vaborbactam [23]. Consequently, MBL-producing CR-Kp are currently more difficult to treat than KPC-Kp [45,46]. In fact, beyond older drugs (e. g., colistin, fosfomycin, tigecycline), only cefiderocol and the combination between aztreonam and CZA are among the potential therapeutic options for MBL-producing strains [45 and references therein], even if additional promising drugs are in advanced stages of the pipeline (e. g., cefepime/taniborbactam, cefepime/zidebactam) [47].

The description of the OXA-48 carbapenemase in 2004 [42] was followed by the detection of several variants, with OXA-181 and OXA-232 rapidly emerging on a global scale [48–50 and references therein].

OXA-48-like enzymes show distinct features in terms of hydrolytic profile. In general, these enzymes efficiently hydrolyze narrow-spectrum β-lactams, while often sparing extended

spectrum cephalosporins and showing only a weak activity toward carbapenems. Indeed, susceptibility to carbapenems and extended spectrum cephalosporins may vary significantly among OXA-48-producing isolates, depending on permeability issues and the co-production of other β-lactamases [49], which may hamper the identification of these resistance mechanisms. Indeed, OXA-48-like enzymes display heterogeneous hydrolytic activity toward carbapenems, e. g., OXA-181 is a stronger carbapenemase than OXA-48, while OXA-232 has a weaker activity and OXA-163 is not attacking carbapenems at all [48].

3. Plasmids involved in acquired resistance to carbapenems

Plasmids play a crucial role in the dissemination of resistance genes in *K. pneumoniae*. The major carbapenemase-encoding genes (i. e., bla_{KPC}-, bla_{NDM}-, bla_{VIM}-, bla_{IMP}- and bla_{OXA-48}-type) are mostly located on plasmids, which facilitate intra- and inter-species horizontal transfer of these resistance determinants. In fact, during the last three decades, acquisition of carbapenemase genes through plasmids has largely contributed to shaping the carbapenemase-producing *K. pneumoniae* (CP-Kp) population worldwide [51–53].

A remarkable variety of plasmid replicons has been reported in CP-Kp, including IncF, C, X3, L, M, N, R, HI1B, and Col (Table 1; for details on bioinformatics methods followed to collect and analyze carbapenemase-encoding plasmids see Supplementary Material M1), underscoring the wide distribution of carbapenemase genes across a variety of plasmid scaffolds. Moreover, several of these carbapenemase-encoding plasmids possess multiple replicons (two or three), which may confer an evolutionary advantage for spreading and maintenance in different bacterial species [54–56] (Tables 1 & S1).

Aboard plasmids, carbapenemase genes are typically associated with mobile genetic elements (MGEs), such as transposons, insertion sequences (IS), and integron-associated mobile gene cassettes, which may contribute to their dissemination among different plasmid platforms and also to plasmid rearrangements, with amplification of the resistance gene dosage (which could have relevant clinical implications in terms of the resulting resistance phenotype) [57]. The aforementioned plasmids, almost invariably, carry other resistance determinants providing additional selective advantages in the clinical setting, and allowing *en-bloc* horizontal transfers of arrays of resistance genes responsible for complex multi-drug resistant (MDR) phenotypes [52,58].

3.1. Genetic structures of carbapenemase-encoding plasmid prototypes and associations of multiple resistance plasmids

3.1.1. IncFII replicons

One of the most prevalent plasmid type carrying variants of bla_{KPC} or bla_{NDM} genes is the IncFII replicon. Additional origins of replication (e. g., IncFIB, IncR and $repB_{R1701}$) commonly co-exist in IncFII plasmids, thus conferring a broader host range [59,60].

The first described IncFII carbapenemase-encoding plasmid, pKpQIL, is a conjugative plasmid with a scaffold similar to pKPN4 (accession no. CP000649) but carrying bla_{KPC-3} (Figure 1). Following first description in a *K. pneumoniae* clinical strain isolated in Israel [59,61], pKpQIL-like plasmids were found associated with the global spread of KPC-2/KPC-3 enzymes among the KPC-Kp clinical populations [51,62–65]. The scaffold of pKpQIL includes genes involved in plasmid transfer, partitioning and stability and carries two replicons, FIB_{pQil} and $IncFII_{pKP91}$. The bla_{KPC-3} gene is bracketed by two IS elements, IS*Kpn7* and IS*Kpn6*, and is part of a Tn*3*-related transposon (Tn*4401*) [66] (Figure 1). Apart from Tn*4401*, the scaffold is punctuated with various IS elements (IS*26*, IS*Kpn14*, IS*Kpn25* and IS*Kpn31*) that may facilitate rearrangements, such as duplications/insertions/deletions, or recombinations with other plasmids [57]. This genetic plasticity has resulted in a number of pKpQIL-like plasmid derivatives possessing two or even three copies of the bla_{KPC} gene, as well as deletions of segments containing other resistance genes, and in the formation of hybrid/chimeric IncFII plasmids encoding KPC-type carbapenemases [64].

A similar $IncFIB_{pQil}$/$IncFII_{pKP91}$ replicon, designated as plasmid p2, was found to encode the NDM-1 MBL [67]. This plasmid type was first isolated in the U.S.A. (from a patient originating from North Africa), but subsequently it has been reported from various countries (Table 1).

Another distinct group of IncFII plasmids possessing $IncFII_{pKP91}$/IncR replicons is strongly associated with KPC and NDM production. This plasmid group is mainly harbored by multilocus sequence type 11 (ST11) CR-Kp strains isolated in China (Table 1), and the main representative is the mosaic pKP048 conjugative plasmid [60]. The bla_{KPC}-containing genetic platform differs from that of Tn*4401* by having a core segment comprising IS*Kpn8*-bla_{KPC-2}-IS*Kpn6*. At the boundaries of this core segment are located a Tn*1721* and a Tn*3* forming, along with the core segment, the Tn*6296* transposon which is one of the major mobile platforms carrying bla_{KPC} in China [60,68]. The pKP048 carries also IS*26* elements as well as additional insertion sequences (IS*4321*, IS*Ec28*, IS*Ec29*, IS*CR1*, IS*Kpn14* and IS*Kpn24*) which are scattered in the plasmid backbone and resistance region (Figure 1).

3.1.2. IncL replicons

IncL replicons belong to the broad host range IncL/M family of plasmids and are associated with the dissemination of OXA-48-like-encoding genes. They display extensive sequence homology with the backbone of other IncL/M plasmids, including the *tra* locus and the partitioning module. Yet, the *traX*, *traY*, and *excA* genes differ significantly [69]. The IncL plasmids have been reported worldwide in *K. pneumoniae* (Table 1) and are linked to the production of OXA-48-type carbapenemases (OXA-48/162/244/517/519). They were also found sporadically associated with bla_{KPC} [69,70].

A prototype of this group is the pOXA-48a plasmid carrying the bla_{OXA-48} gene embedded in a Tn*1999* mobile element (Figure 1), inserted within the *tir* locus encoding a transfer inhibition protein. Interestingly, the insertional inactivation of *tir* by Tn*1999* has been associated with a higher conjugal transfer frequency of plasmid pOXA-48a [71]. This genetic

Table 1. Analysis of complete carbapenemase encoding plasmids described in *K. pneumoniae* and available from INSDC databases (accessed on June 2023). Plasmids are grouped by distinct replicon type(s)/carbapenemase gene(s). Only combinations reported from at least three different countries are shown. For each combination, the prevalence of 16S ribosomal methylases conferring pan-aminoglycoside resistance is also shown. The presence of multiple copies of a carbapenemase-encoding gene or the simultaneous presence of different cabapenemase is also reported. KPC and NDM variants conferring resistance to ceftazidime/avibactam or cefepime/taniborbactam, respectively, are marked with a star. IMP β-lactamases are marked with a star to indicate cefepime/taniborbactam resistance.

Replicon(s) (No of plasmids)	Size Range (Kbp)	Carbapenemase gene(s)	Multiple carbapenemases	16S ribosomal methylases (%)	Countries	Year of First detection	Prototype[1] (Accession)
FII_{pHN7A8}, R (316)	58–279	$bla_{KPC-2/17/33*/71*/74*/78*/79*/93}$	Two copies ($n = 12$) Three copies($n = 2$)	*rmtB1* (75.0%)	Canada, China, Egypt, Taiwan	2010	pXHKP6–1 (CP066888.1)
FII (44)	75–103	$bla_{NDM-1/5/16b}$		*rmtB1* (86.36%)	Bangladesh, Czech Republic, India, Myanmar, Nepal, Russia, South Korea, Thailand, United Arab Emirates, USA	2013	pABC143C-NDM (KY130431.1)
FIB_{pQil}, FII_{pKP91} (139)	66–143	$bla_{KPC-2/3/29*/31*/49*/53*/66*/67*/69*/70*/110/121/125/136}$	Two copies ($n = 5$) Three copies($n = 1$)		Australia, Canada, Czech Republic, France, Israel, Germany, Greece, Hong Kong, Italy, Norway, Poland, Russia, Switzerland, Taiwan, United Kingdom, USA	2003	p13001-QIL2 (CP098358.1)
FIB_{pQil}, FII_{pKP91} (26)	96–192	$bla_{NDM-1/5}$	NDM-5/OXA-232 ($n = 2$)	*rmtB1* (15.38%) *rmtF1* (42.31%)	Bangladesh, China, Egypt, Thailand, United Arab Emirates, United Kingdom, USA	2012	p2 (CP009115.1)
FIB_K, FII_{pKP91} (29)	131–242	$bla_{KPC-2/3/4}$			Australia, China, Denmark, France, Greece, Italy, USA	2009	pBIC-1a (CP022574.1)
FIB_K, FII_{pKP91} (7)	142–583	$bla_{NDM-1/7}$	NDM-1/SFO-1 ($n = 1$)	*armA* (28.57%) *rmtF1* (28.57%)	Bangladesh, China, Egypt, India, South Africa, USA	2015	pEGKP4.2 (CP048802.1)
FII_{pKP91}, R (16)	87–151	$bla_{KPC-2/3/111}$		*armA* (25.0%)	China, Czech Republic, Italy, USA	2006	pKp048 (FJ628167.2)
FII_{pKP91}, R (7)	107–155	$bla_{NDM-1/5}$		*rmtB1* (42.86%)	Bangladesh, Egypt, Hong Kong	2015	pEGKP3.3 (CP046647.1)
FII_{pKP91}, $repB_{R1701}$ (18)	69–166	$bla_{KPC-2/3}$			Canada, China, Hong Kong, Japan, Portugal, Singapore, Sweden, USA	2009	pHS091147 (KX236178.1)
FII_{pKP91}, $repB_{R1701}$ (11)	106–368	$bla_{NDM-1/4}$		*rmtF1* (9.09%)	China, Taiwan, Viet Nam	2013	pKp21774–135 (MG878868.1)
$FII_{pBK30683}$, $repB_{R1701}$ (41)	83–165	$bla_{KPC-2/3/33*/112*/136}$			China, Taiwan, USA	2015	pSZF_KPC (MH917122.1)
FIB_{pB171}, FII_{Yp} (22)	86–120	bla_{NDM-1}		*rmtC* (90.91%)	Canada, China, Germany, Romania, South Africa, United Kingdom, USA	2012	pKp199–2 (CP035537.1)
FII_{pHN7A8} (36)	37–180	$bla_{KPC-2/12/33*}$	Two copies ($n = 2$)	*rmtB1* (69.44%)	Canada, China, Taiwan	2012	unnamed2 (CP023938.1)
R (41)	47–173	$bla_{KPC-2/3/12/33*}$	Two copies ($n = 1$) Five copies ($n = 1$)	*rmtB1* (12.2%)	China, Czech Republic, Egypt, Taiwan, USA	2010	p34618–71.572kb (CP010396.1)
R (4)	42041–152803	bla_{NDM-1}		*armA* (50.0%) *rmtF2* (25.0%)	Bangladesh, Italy, Singapore	2014	pSg1-NDM (CP011839.1)
C (13)	120–210	$bla_{KPC-2/33*}$		*armA* (7.69%)	Canada, China, USA	2010	pKPC_CAV1344 (CP011622.1)
C (59)	72–213	$bla_{NDM-1/5}$	NDM-1+SFO-1 ($n = 4$)	*armA* (18.64%) *rmtB1* (1.69%) *rmtC* (32.2%)	Bangladesh, Chile, China, Colombia, Hong Kong, Kenya, Myanmar, Poland, Russia, Sweden, Switzerland, Thailand, USA	2010	pNDM-US (CP006661.1)
C (4)	117–178	$bla_{OXA-48/181/567}$		*armA* (50.0%)	Argentina, Germany, Switzerland	2014	unnamed2 (CP017987.1)
$FIB_{pNDM-Mar}$, $HI1B_{pNDM-MAR}$ (50)	235–377	$bla_{NDM-1/5/7/29}$	Three copies ($n = 1$)	*armA* (74.0%) *rmtF1* (6.0%)	Bangladesh, Czech Republic, Egypt, Germany, India, Italy, Korea, Nepal, Peru, Poland, Qatar, Russia, Saudi Arabia, South Korea, Switzerland, Thailand, United Kingdom, USA	2009	pKp46596–1 (CP059311.1)
$FIB_{pNDM-Mar}$, $HI1B_{pNDM-MAR}$, R (4)	320–423	$bla_{NDM-1/5}$		*armA* (50.0%)	Pakistan, Russia, USA	2016	pKP_160–2 (CP078034.1)

(*Continued*)

Table 1. (Continued).

Replicon(s) (No of plasmids)	Size Range (Kbp)	Carbapenemase gene(s)	Multiple carbapenemases	16S ribosomal methylases (%)	Countries	Year of First detection	Prototype[1] (Accession)
L (261)	31–97	$bla_{\text{OXA-48/162/244/517/519}}$	Two copies ($n = 2$)	*rmtF1* (0.38%)	Australia, Belgium, China, Ecuador, Egypt, France, Germany, Ghana, Greece, India, Lebanon, Netherlands, Poland, Qatar, Russia, Spain, Sweden, Switzerland, Taiwan, Turkey, United Kingdom, U.S.A., Viet Nam	2001	pOXA-48a (JN626286.1)
N (29)	38–84	$bla_{\text{KPC-2/3/4/14*/136}}$	Two copies ($n = 1$)		Brazil, Chile, China, Germany, Korea, United Kingdom, USA	2003	pBK13048-KPC14 (CP045022.1)
N (30)	39–61	$bla_{\text{IMP-1*/4*/6*/8*}}$			China, Japan, Taiwan	2009	pD610-1IMP (MK036890.1)
N (7)	37–63	$bla_{\text{NDM-1/6}}$			China, Taiwan	2016	p4 (CP026590.1)
M2 (7)	76–91	$bla_{\text{NDM-1}}$		*armA* (71.43%)	Italy, Myanmar, Oman, United Kingdom	2014	pIT-Kpn-01/2014 (MH722217.1)
FIB_{pQil} (5)	78–122	$bla_{\text{KPC-2/3/31*}}$			Brazil, Greece, Italy	2000	unnamed1 (CP018884.1)
FIB_{pQil} (19)	46–138	$bla_{\text{NDM-1}}$		*rmtF1* (15.79%)	Bangladesh, Hong Kong, Italy, Myanmar, Poland, Switzerland, Thailand, United Kingdom, USA	2014	pNDM-1fa (CP014757.1)
FII_{pKPX1}, $repB_{R1701}$ (7)	77–138	$bla_{\text{NDM-1}}$		*rmtF1* (14.29%)	India, Poland, Switzerland	2012	pIncFII_6713 (MT415054.1)
X3 (37)	13–67	$bla_{\text{KPC-2/3/55}}$	Two copies ($n = 1$)		Brazil, France, Germany, Italy, Russia, South Korea, United Arab Emirates, USA	2009	pIncX3 (CP080700.1)
X3 (110)	18–93	$bla_{\text{NDM-1/4/5/6/7/19/33}}$	Five copies ($n = 1$)		Australia, Bangladesh, Canada, Chile, China, Egypt, India, Jamaica, Myanmar, South Korea, United Arab Emirates, Viet Nam	2010	pABC52-NDM-1 (MK372381.1)
M1 (4)	67–89	$bla_{\text{KPC-2/3}}$	KPC-2 + NDM-1 ($n = 1$)		Brazil, China, USA	2008	pKPC_CAV1042–89 (CP018669.1)
M1 (16)	30–75	$bla_{\text{OXA-48}}$			Germany, Netherlands, Saudi Arabia, Switzerland	2013	pKp_Goe_070–2 (CP018452.1)
FIA_{HI1}, R (4)	74–346	$bla_{\text{NDM-1/9*}}$			Poland, Switzerland, Viet Nam	2012	pIncR_6713 (MT415057.1)
ColKP3, X3 (22)	50–70	$bla_{\text{OXA-181}}$	OXA-181 + NDM-5 ($n = 1$)	*rmtB1* (4.55%)	China, Czech Republic, India, Italy, Netherlands, South Korea, Thailand, United Arab Emirates	2013	pBC947-OXA-181 (MK412920.1)
ColRNAI (22)	16–65	$bla_{\text{KPC-2/3}}$			China, Colombia, Italy, Japan, Switzerland, USA	2005	pNJST258C2 (CP006919.1)
X5 (6)	22–48	$bla_{\text{KPC-2/3}}$			Brazil, China, Taiwan, USA	2006	p13190–3 (CP026020.1)
FII_S (4)	75–86	$bla_{\text{NDM-1/4}}$		*rmtB1* (100.0%)	China, South Korea, Viet Nam	2015	pMH15-208H_1 (AP018580.2)
N2 (10)	38–52	$bla_{\text{NDM-1}}$			China, Thailand, USA	2015	pC057_NDM1 (LC521837.1)
ColKP3 (99)	3–51	$bla_{\text{OXA-181/232}}$			Bangladesh, China, Czech Republic, Germany, Hong Kong, India, South Korea, Netherlands, Niger, Pakistan, Switzerland, Thailand, United Arab Emirates, USA	2011	pABC120-OXA (MF774791.1)
ColKP3, ColKP3 (5)	10–12	$bla_{\text{OXA-232}}$	Two copies ($n = 5$)		Canada, China, India	2015	unnamed3 (CP023924.1)

[a]Prototype plasmids are chosen according their isolation date.
[b]p13001-QIL has been selected as prototype of pKpQIL-like plasmids because the former has been detected in a isolate obtained in 2003.

configuration has been deemed as a key factor contributing to the successful dissemination of pOXA-48a at a global scale. Intriguingly, in the case of this prototype plasmid, no other resistance genes and mobile elements have been identified. The same applies to the majority of plasmids of this type [70].

3.1.3. IncX3 replicons

Several plasmids harboring bla_{NDM} or bla_{KPC} belong to the IncX3 subgroup of IncX replicons. These plasmids have a high efficiency of conjugal transfer and have disseminated in Asian, European, and South American countries (Table 1). The prototype of this group is the NDM-1 encoding pABC52-NDM-1 plasmid which was found in an ST11 *K. pneumoniae* clinical isolate from the United Arab Emirates [72] (Figure 1). The plasmid backbone contains intact conjugal transfer and partitioning regions. Two β-lactamase genes are present on pABC52-NDM-1, bla_{NDM-1} and bla_{SHV-12}. The bla_{NDM-1} gene is followed downstream by the bleomycin resistance gene ble_{MBL}, and this genetic segment is surrounded by truncated IS*Aba125* and IS*CR27* elements. In turn, this region is flanked by a Tn*3* and an IS*26* element. The latter is located at the edge of the IS*26* composite transposon carrying the bla_{SHV-12} gene. A third IS*26* element is located upstream bla_{NDM-1}. Therefore, a novel putative IS*26* composite transposon carrying all resistance genes is formed on pABC52-NDM-1, providing an opportunity for further mobilization of this segment to other plasmids. Several similar IncX3 plasmids have been reported carrying bla_{NDM} (one to five copies in tandem) and bla_{KPC} variants (Table 1).

3.1.4. IncC replicons

IncC is yet another group of plasmids that significantly contributed to the dissemination of carbapenemase-encoding genes among *K. pneumoniae* and other Enterobacterales. The group comprises plasmids with extended resistomes including genes for various carbapenemases such as NDM, KPC, IMP and VIM (Table 1 and Table S1). The prototype of this group is considered the pNDM-US plasmid [73] (Figure 1). Aboard of this element, bla_{NDM-1} is part of a ΔTn*125* transposon with the array of ΔIS*Aba125*/bla_{NDM-1}/ble_{MBL} core region. The plasmid carries also bla_{CMY-6} as part of an IS*Ecp1*-derived transposon inserted in the *tra* region and also containing the *blc* and *sugE* genes from *Citrobacter freundii*. This genetic structure is preceded by a ΔTn*1696* transposon and the In*46* integron containing the *aac4* and *sul1* genes. IS*Kpn14*, IS*4321* and IS*3000* are also located on the plasmidic scaffold.

3.1.5. ColKP3 replicons

The ColKp3 replicon, that belongs to the ColE plasmid family, is strongly associated with the $bla_{OXA-181/232}$ variants (Table 1). The archetype for this group is the OXA-232-encoding pABC120-OXA plasmid first reported in 2011 in the United Arab Emirates [74]. pABC120-OXA is a small non-conjugative plasmid which includes a *mob* region encoding proteins for the mobilization process (Figure 1). $bla_{OXA-232}$ is the sole resistance gene identified on the replicon and appears as a part of a truncated-Tn*2013* transposon containing only the 3′ end of the IS*Ecp1* element.

3.1.6. Association of multiple plasmids

K. pneumoniae clinical strains very frequently carry multiple resistance plasmids, with carbapenemase-encoding replicons often co-existing with plasmids of different incompatibility groups carrying other types of carbapenemases, ESBL determinants or genes conferring resistance to other classes of antibiotics (i. e., to aminoglycosides, fluoroquinolones, colistin, sulfonamides, trimethoprim, chloramphenicol, and macrolides). As a consequence, CP-Kp isolates can exhibit expanded resistomes leading to multi- or pan-resistant phenotypes and difficult to treat infections. For example, a KPC-producing *K. pneumoniae* strain of ST258 described in Italy harbored four plasmids, one carrying bla_{KPC-3} plus other three with a different set of genes contributing to a complex MDR phenotype [75]. Another paradigm is represented by an ST11 *K. pneumoniae* strain from China equipped with four plasmids, in which the co-production of the KPC and NDM carbapenemases together with other 38 plasmid-encoded resistance factors has been observed [76]. In addition, a *K. pneumoniae* isolated in South Korea [77] harbored three different plasmids: two of them encoded carbapenemases (NDM-1 and OXA-232) and the third one the CTX-M-15 ESBL, along with several other resistance determinants. It has been proposed that the co-existence of the NDM-1 and OXA-232 plasmids is able to confer, in addition to an extended resistome, a higher fitness and virulence leading to dissemination of these strains even in the absence of selective pressure [**77**].

4. Population structure of carbapenem-resistant *K. pneumoniae*: diversity and role of high-risk clones

The emergence and dissemination of successful *K. pneumoniae* epidemic clones, globally referred as 'high-risk clones' (HiRiCs), are major drivers of carbapenem resistance spread and of carbapenemase genes dispersal. Since their first definition [78], such clonal groups (CG), often represented by few successful STs, experienced a global dissemination and exhibited a remarkable propensity toward the acquisition of multiple resistance determinants, including those for major carbapenemases, mostly mediated by the recruitment of successful and epidemic plasmids. The different CGs display important geographical heterogeneity in their prevalence, contribution to MDR infections and carriage of carbapenem resistance traits, with some of them being described as strictly linked with few resistance genes (e. g., bla_{KPC}-type carbapenemases), while others being associated with a broader spectrum of determinants [52,79].

HiRiCs include the well-studied and widely geographically distributed CG11, CG15, CG17, CG20, CG29, CG37, CG101, and CG258, and the more recently emerged CG147 and CG307 [80,81]. Among these, CG258, mostly consisting of two single locus variant STs (namely ST258 and ST512), and CG11 represent the most diffused and successful clones of CR-Kp, being responsible of large nosocomial outbreaks worldwide and endemic dispersal [44] and references therein,[80]. These CGs are characterized by different geographical prevalence [**79** and references therein], with CG258 being predominant in the Americas, southern Europe and some regions of Asia such as Japan and Middle East, and CG11 prevailing in some

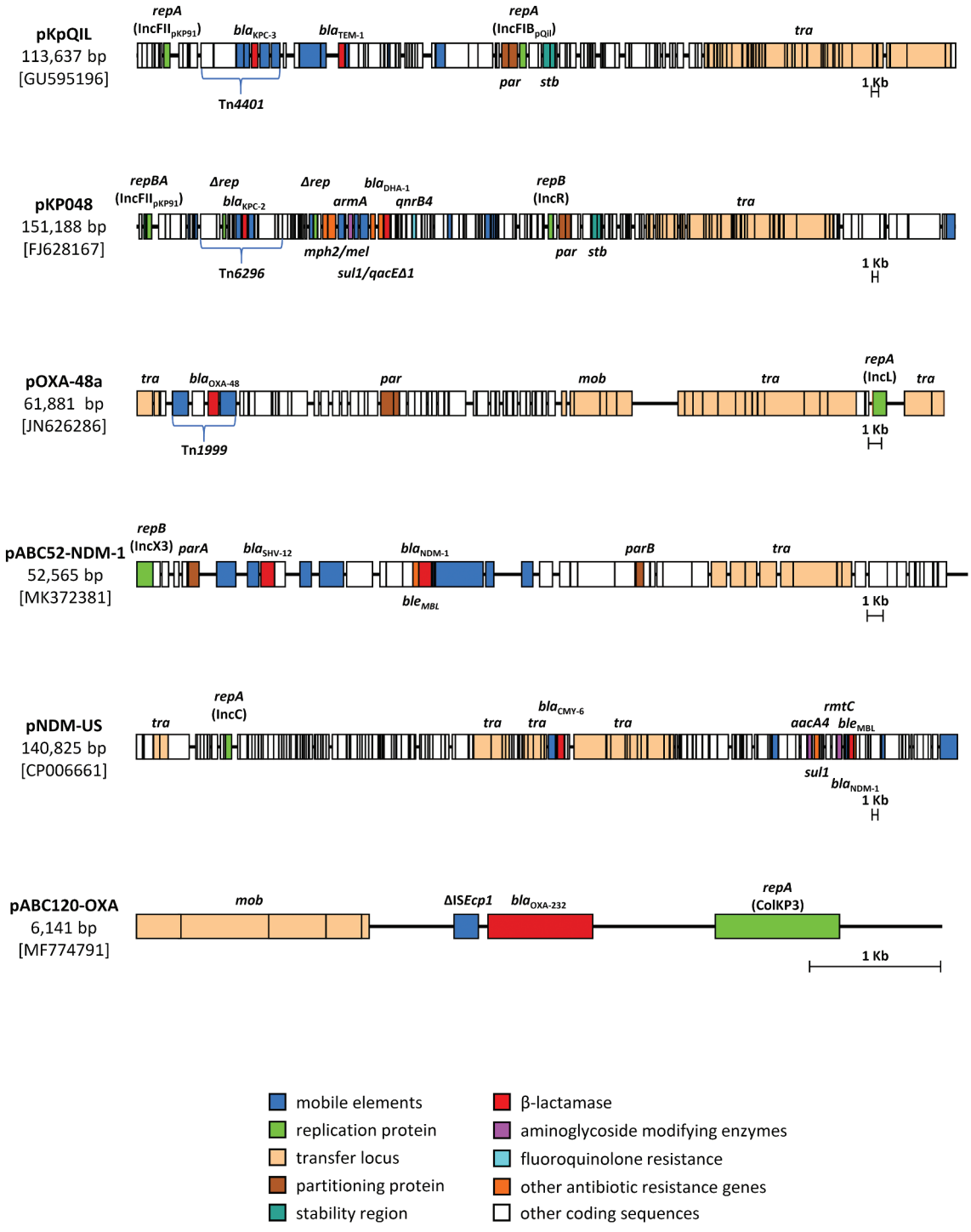

Figure 1. Schematic representation of genetic structures of carbapenemase-encoding *K. pneumoniae* prototype plasmids. IncFIB$_{pQil}$/IncFII$_{pKP91}$ pKpQIL (GU595196), IncFII$_{pKP91}$/IncR pKP048 (FJ628167), IncL pOXA-48a (JN626286), IncX3 pABC52-NDM-1 (MK372381), IncC pNDM-US (CP006661) and ColKP3 pABC120-OXA (MF774791).

parts of South America (e. g., Brazil) and Asia (e. g., East Asia and China) (Figure 2). CG15, CG17, CG101, CG147, and CG307 are less diffused, with some of them (e. g., CG15, CG17, and CG101) being also traditionally associated with the spread of ESBL-type determinants [52]. However, CG147 and CG307 have emerged globally since the late 2000s as important vehicles for the dissemination of carbapenemase genes, becoming prominent global clones in some geographical areas (e. g., southern Europe, the Indian subcontinent, North Africa, and Middle East) [82] (Figure 2).

Since the first description of nosocomial outbreaks sustained by KPC-type carbapenemase producers, KPC-Kp population structure appears to be dominated by the clonal expansion of few extremely successful clones [31,78], such as CG258 and CG11, that became rapidly endemic in many geographic areas (Figure 2); however, in the following years, KPC-producing *K. pneumoniae* epidemiology changed toward a more polyclonal scenario, with novel emerging clones (such as ST101 and ST307) outcompeting CG258, particularly in Europe [52,83–85]. Of note, *K. pneumoniae* strains belonging

in CG258, which played a major role in the early global emergence and dissemination of KPC-type enzymes [31,53], have been almost stably associated with IncFIB$_{pQil}$/IncFII$_{pKP91}$ plasmids (e. g., the pKpQIL plasmid), that first appeared within this clonal lineage [59] (Figure 3 and Table S2). Indeed, IncFIB$_{pQil}$/IncFII$_{pKP91}$ plasmids appear to be only sporadically described in other emerging KPC-producing CGs, such as CG147 and CG307, that conversely harbor this determinant associated with a wider range of replicon types (e. g., IncFIB$_K$/IncFII$_{pKP91}$, IncC, and IncR plasmids). In CG11, KPC dissemination is prevalently linked to IncFII$_{pHN7A8}$ plasmids, often equipped with other replicons (e. g., IncR replicon); such plasmids are almost exclusively described in China, consistently with the wide diffusion of CG11 in this geographic area [79 and references therein].

NDM-producing *K. pneumoniae* strains are distributed across a vast number of CGs, suggesting that no obvious HiRiC can be recognized as responsible for the dissemination of this determinant. However, NDM-producing CG11, CG15, and CG147 strains are relatively common lineages that have been reported globally [86], mostly carrying *bla*$_{NDM}$ genes on IncX3, IncC, and IncFII plasmids. In this polyclonal scenario, CG147 likely represents the most prominent epidemic clone currently mediating the international spread of *bla*$_{NDM}$ in the Middle East, the Indian subcontinent, Europe and the Americas [82,87], also causing large regional outbreaks [88,89].

Differently from what has been observed for the global success of KPC-type-harboring CG258 and CG11, diffusion of *bla*$_{OXA-48}$-like determinants appears to be rather linked to a polyclonal dissemination and to successful plasmids with high spreading ability, such as ColKP3 and IncL plasmids. Nevertheless, certain *K. pneumoniae* HiRiCs (e. g., CG11, CG15, CG101, CG147, CG231, and CG307) have been associated with the global dispersion of OXA-48-like carbapenemases [90,91], although with significant geographical differences. As an example, *bla*$_{OXA-48}$ determinants dominating the European scenario are mainly associated to CG11 and CG147 strains harboring IncL plasmids, while in East Asia and in the Indian subcontinent CG231 strains account for the majority of *bla*$_{OXA-232}$-positive *K. pneumoniae* (Figure 2) [92,93]; in these isolates, ColKP3 plasmids represent the dominant structure responsible for OXA-232 dissemination (Figure 3). Of note, a significant proportion of CG11 and CG147 OXA-232-producing *K. pneumoniae* (and to a lesser extent of OXA-181 producers) also co-produce NDM-1, further contributing to worsen their complex resistance profile [94–97].

5. Resistance accretion and modulation by plasticity of carbapenemase-encoding plasmids: paradigms of different epidemiological successes

The presence of multiple plasmid-encoded antibiotic resistance genes is frequently observed in contemporary clinical isolates of several Gram-negative species [98], appearing to be a rule more than an exception in *K. pneumoniae*. In particular, in this species, as well as in other Enterobacterales, carbapenemase-encoding plasmids often possess additional β-lactamases as well as resistance factors to other antibiotic classes, therefore acting as genetic platforms able to confer complex MDR phenotypes by single transfer events (Table 1).

For example, pKpQIL, the prototype IncFIB$_{pQil}$/IncFII$_{pKP91}$ *bla*$_{KPC-3}$-positive plasmid, carried also *bla*$_{TEM-1}$ and inactive *bla*$_{OXA-9}$ and *aadA* alleles [52,59,61]. By a bioinformatics analysis of complete carbapenemase-encoding plasmids available through the National Center for Biotechnology Information web-site (Supplementary Material M1), complemented with literature search, it can be observed that pKpQIL-like plasmids equipped with the same basic set of resistance genes, involved in different stages of KPC-Kp pandemic, have been described in several countries including the U.S.A. [62], UK [99], Greece, Poland [51], Brazil [100], Norway [101], Italy [64], China [65], and Taiwan [52,102]. In some cases, these replicons carried an expanded resistome including also genes coding for aminoglycoside modifying enzymes and for resistance to trimethoprim/sulfamethoxazole [75,98,103–105]. Lately, IncFIB$_{pQil}$/IncFII$_{pKP91}$ plasmids with *bla*$_{NDM-1}$, *bla*$_{NDM-5}$ or *bla*$_{OXA-232}$, and characterized by different arrays of quinolone, aminoglycosides, rifampicin, and macrolide resistance genes have also been described [67,106,107], underscoring the plasticity and evolutionary potential of such elements. In these plasmids *bla*$_{NDM-1}$ is frequently associated with the *bla*$_{CTX-M-15}$ ESBL gene, quinolone and aminoglycoside resistance genes (e. g., *qnrS1/qnrB1* and *aac(3')-VI/aac(6')-Ib* variants and *rmtF1*, respectively) [67,108,109], while *bla*$_{NDM-5}$ has been found together with aminoglycoside, chloramphenicol and trimethoprim/sulfamethoxazole resistance genes (e. g., *rmtB1* together with *rmtF1, catA1/catB* and *dfrA12/sul1*, respectively) [110], and with the *bla*$_{SHV-12}$ ESBL, rifampicin, chloramphenicol, aminoglycosides, and trimethoprim/sulfamethoxazole resistance genes, even in combination with *bla*$_{OXA-232}$. It is worth noting that the presence of genes encoding the RmtF or RmtB 16S rRNA methylases, often observed in IncFIB$_{pQil}$/IncFII$_{pKP91}$ NDM-positive plasmids, is associated with a pan-aminoglycoside resistance phenotype including the recently approved aminoglycoside plazomicin [111].

Very often, MDR plasmids evolve over time through large genetic rearrangements, creating hybrid structures composed not only by multi-replicons, but also exhibiting complex resistance genes arrangements. As an example, *bla*$_{KPC}$ genes have been found to be located either on several Tn*4401* isoforms or on different genetic structures which are associated to 119 single replicons or replicons combinations (Table 1 and Table S1). Similarly, *bla*$_{NDM}$, and *bla*$_{OXA-48}$-like genes have been found located on plasmids belonging to more than 70 and 35 single or multiple incompatibility groups, respectively. However, despite this remarkable genetic diversity, in several cases a restricted number of plasmid structures are often found in *K. pneumoniae* clinical isolates from different countries and, on some occasions, these latter replicons experienced an extraordinary epidemiological success. For example, KPC-encoding IncFII$_{pHN7A8}$/IncR plasmids frequently encoding CTX-M-15, SHV or TEM β-lactamases, as well as RmtB, have been repeatedly reported in China from 2010 and sporadically in other countries including Egypt [112] and Taiwan [113]. IncFII$_{pHN7A8}$/IncR plasmids, the most represented structures in public nucleotide databases by far, are strictly associated with a single type of carbapenemase gene, i. e., a *bla*$_{KPC}$ allele, and have been described mostly in CG11 isolates (Figure 3).

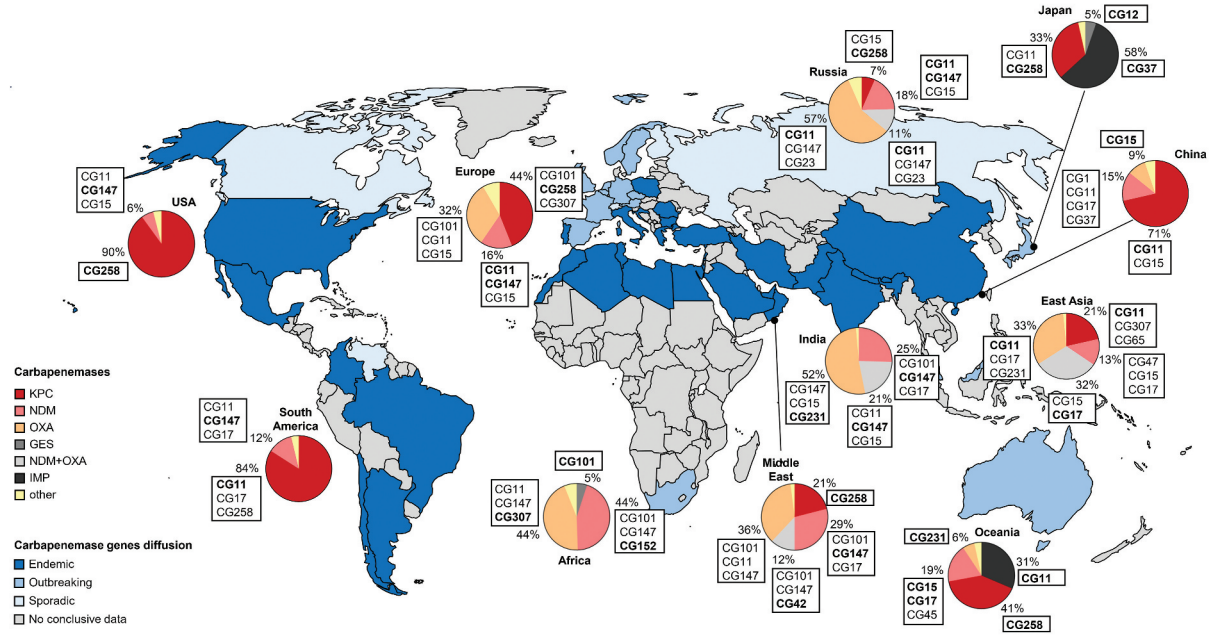

Figure 2. Geographical distribution and prevalence of carbapenemases genes among *K. pneumoniae*. Graphs were obtained using data from publicly available genomic sources https://www.ncbi.nlm.nih.gov/nucleotide/, last accessed on June 2023). Areas with endemic, outbreaking or sporadic description of carbapenemases-producing strains are shown. Prevalence of the different carbapenemases genes is reported as percentage over total number of records for each geographical area; prevalences > 5% are detailed in the graph. For each determinant, the three most frequent CGs associations are indicated. Only CGs accounting for > 5% of the strains are reported and CGs covering ≥ 25% of the carbapenemases-producing strains are shown in bold.

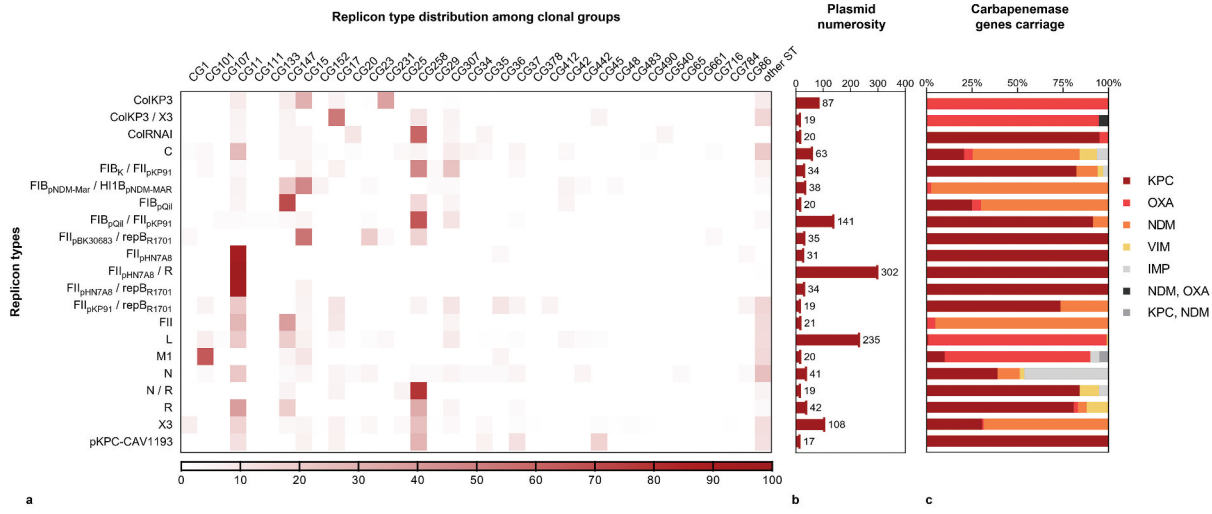

Figure 3. **Panel A.** Heatmap showing prevalence of plasmids ($n = 1,346$) harboring different replicon types among *K. pneumoniae* CGs, expressed as percentage of occurrence of each replicon type among CGs over total number of each category of plasmids (data source: https://www.ncbi.nlm.nih.gov/nucleotide/, last accessed August 28, 2023). Only replicon types occurring with a frequency ≥ 1% are shown. **Panel B.** Numerosity of each replicon type group of plasmids. **Panel C**. Prevalence of carbapenemase determinants and their combinations (%) among each plasmid group.

KPC- or NDM-encoding IncFII$_{pKP91}$/IncR MDR-plasmids have been described in China but also in Europe [114,115], Northern Africa, Bangladesh, and U.S.A.. In some cases (e. g., plasmids pKP048 and pNDM-US), AmpC-type β-lactamase genes (*bla*$_{DHA-1}$, *bla*$_{CMY-6}$) and a variety of other resistance determinants (*armA, rmtC, aacA4, mph(E), msr(E), qnrB4, sul1*), conferring resistance to several antibiotic classes, were also present [60,73].

IncL plasmids carrying *bla*$_{OXA-48}$ or IncC replicons positive for NDM-1, aminoglycoside, chloramphenicol, and trimethoprim/sulfamethoxazole resistance genes, have experienced a world-wide dissemination. Description of related plasmid backbones in multiple continents has also been reported for: i) KPC- or NDM-encoding IncX3 plasmids; ii) OXA-181/OXA-232-encoding ColKP3 plasmids, where non-β-lactam resistance genes were mostly absent; iii) IncN plasmids encoding KPC

and NDM-positive IncFII replicons, both mostly associated to aminoglycoside, chloramphenicol, and trimethoprim/sulfamethoxazole resistance genes; iv) IncR KPC-producing plasmids equipped with aminoglycoside and chloramphenicol resistance genes and v) IncHI1B$_{pNDM-MAR}$/IncFIB$_{pNDM-Mar}$ NDM plasmids carrying aminoglycoside, quinolone, and chloramphenicol resistance genes. It is interesting to underscore that in some carbapenemase-positive plasmids (e. g., those having IncL and ColKP3 replicons), a single resistance factor is frequently found (i. e., a *bla*$_{OXA-48}$-like gene). A similar finding is also observed for IncX5 plasmids, where *bla*$_{KPC}$-type β-lactamases are the only resistance gene detected.

Beyond these examples of genetic linkage of multiple resistance genes in single plasmids (i. e., accounting for resistance accretion), which have led to the epidemiological success of some elements, plasmids can further provide their hosts with the ability to evolve alternative antimicrobial resistance strategies. Accordingly, plasmids can act as drivers of rapid phenotypic changes, playing a primary role in resistance modulation other than accretion.

This concept is well exemplified by the recent description of KPC-Kp resistant to novel BLICs, such as CZA, meropenem/vaborbactam (MVB) and imipenem/relebactam (IMR), which represented a major breakthrough in the treatment of some infections caused by carbapenem-resistant Enterobacterales (CRE) [23,116]. Several reports highlighted how an increased number of KPC-encoding plasmids per cell following alterations of the *repA2* gene, which encodes a factor involved in plasmid maintenance and replication functions, resulted in resistance to CZA and MVB [117,118]. A consequence of this copy-number variation strategy is that any plasmid-borne resistance genes, such as *bla*$_{KPC}$ in the aforementioned cases, will also be multicopy, leading to potentiation of the resistance phenotype through a greater gene expression.

Consequently, even if KPC-2 or KPC-3 variants do not confer resistance to novel BLICs when produced at basal levels, the variation of the *bla*$_{KPC}$ gene dosage observed upon multimerization of the *bla*$_{KPC}$-harboring transposon Tn*4401* (e. g., multiple copies aboard on the same plasmid or on different elements) was consistently associated with a gain in resistance to CZA and MVB, but not IMR [103]. Interestingly, depending on the magnitude of the *bla*$_{KPC}$ gene dosage, which can be contributed by an increased number of KPC-encoding plasmid per cell, by duplication of Tn*4401* or by combination of both mechanisms, cross-resistance to CZA, MVB, and IMR can be observed among KPC-Kp [103].

6. Evolution of hybrid plasmids encoding resistance and virulence traits

From a clinical standpoint, *K. pneumoniae* can be considered a successful and highly versatile human pathogen, capable of causing both HCAIs and community-acquired infections (CAIs) that could greatly differ in their presentation and severity [10,119]. While opportunistic HCAIs usually affect vulnerable patient groups (e. g., neonates, elderly) with comorbidities, who are immunocompromised and/or have barriers impairment (e. g., intravascular devices, endotracheal tube, or surgical wound), CAIs are often diagnosed in otherwise healthy individuals of any age, who do not share the risk factors for HCAIs (e. g., intestinal carriage), showing a propensity to present with a rapidly progressing invasive disease (e. g., pyogenic hepatic and splenic abscesses, septic endophthalmitis with subsequent metastatic spread), which is an uncommon trait of *K. pneumoniae* and other enterics [10,120,121].

These clinical presentations largely reflect the existence of two *K. pneumoniae* pathotypes, represented by 'classic' (cKp) and hypervirulent (hvKp) *K. pneumoniae* (Figure 4), as results of two distinct evolutionary trajectories of the species [10,119,122].

Traditionally, cKp are well known for their ability to accumulate resistance, which led to the emergence of MDR-Kp, while features of hvKp are less well defined, but most commonly include high invasiveness and susceptibility to multiple antibiotics [10].

MDR-Kp and hvKp, however, present additional significant differences at epidemiological and genetic level (Figure 4). Owing to the emergence of diverse successful HiRiCs (e. g., ST11, ST258/512, ST147, and ST307), MDR-Kp have spread globally and extensively acquired resistance plasmids encoding multiple resistance determinants, as observed with ESBL- and/or CP-Kp [53]. Conversely, hvKp became prevalent in the Asian-Pacific region, where they first emerged in mid-1980 and were linked to a high prevalence of hypervirulent disease [123,124], even though hvKp are increasingly reported worldwide nowadays [**53**,124].

A hallmark feature of hvKp is represented by the carriage of large, typically non-conjugative, plasmids harboring genes related to increased virulence, as observed with the prototype IncHI1B$_{pNDM-MAR}$/repB$_{KLEB_VIR}$ virulence plasmid pLVPK (from *K. pneumoniae* strain CG43, ST86, K2), and the cognate pK2044 (from *K. pneumoniae* strain NTUH-K2044, ST23, K1) (Figure 4) [125,126]. These plasmid-borne virulence factors typically consist in: i) regulators of the mucoid phenotypes (RmpADC, RmpA2) and ii) siderophore systems (i. e., aerobactin, salmochelin, yersiniabactin), specialized iron chelators contributing to the high pathogenicity of hvKp under iron-limiting conditions (Figure 4) [127].

Despite MDR-Kp and hvKp have been historically regarded as well segregated pathotypes, this distinction has become less evident in more recent years due to the convergence of their plasmidomes, leading to the emergence of strains simultaneously exhibiting hyper-resistance (i. e., carbapenem resistance) and hyper-virulence traits [122]. The emergence of convergent strains poses a significant public health threat, and it has been to date reported in Asia, Europe, North, and South America [128]. Several studies employing whole-genome sequencing to perform an in-depth characterization of bacterial strains showed that this convergence can be overall attributed to three evolutionary routes (Figure 4): i) acquisition of resistance plasmids, or other mobile genetic elements, carrying multiple resistance genes (primarily *bla*$_{KPC-2}$ and *bla*$_{NDM-1}$) by typical hvKp lineages, evolving into hvKp^{MDR} [129–131]; ii) acquisition of virulence plasmids by MDR-Kp lineages, evolving into MDR-Kp^{hv} [89,132–135]; iii) emergence of hybrid plasmids (p$^{MDR:hv}$), as a result of recombination between canonical resistance and virulence plasmids from MDR-Kp and hvKp, respectively [128,136–140].

The latter trajectory has gained major attention for three reasons, at least. First, genomic investigations suggested that MDR-Kp are more prone to acquire virulence genes than hvKp are to acquire resistance genes [80], so that the emergence and spread of MDR-Kp^{hv} lineages equipped with p$^{MDR:hv}$ in the clinical setting could account more frequently for severe diseases. Second, the carriage of resistance and virulence determinants in a single mosaic plasmid enables the co-selection of both factors by a single transfer event; this raises an intriguing issue in that the extensive use of antimicrobial agents in the clinical setting could positively select for *K. pneumoniae* lineages carrying virulence determinants. Lastly, the emergence of p$^{MDR:hv}$ plasmids encoding carbapenem resistance has been increasingly reported from 2016 onward [89,124,127,141,142]. This phenomenon appears primarily mediated by the spread of hybrid IncFIB$_{pNDM-Mar}$/IncHI1B$_{pNDM-MAR}$ plasmids, and of IncHI1B$_{pNDM-MAR}$/repB$_{KLEB_VIR}$ or IncHI1B$_{pNDM-MAR}$ to a lesser extent, encoding aerobactin, regulators of the mucoid phenotype, the ArmA or RmtB 16S rRNA methylases together with NDM (i. e., NDM-1, NDM-5, NDM-29), KPC (i. e., KPC-2) or OXA-48-like (i. e., OXA-48, OXA-181, OXA-232) carbapenemases. These elements have been detected in *K. pneumoniae* from UK (2016–2018), Qatar (2016), China (2017–2022), Russia (2017–2021), Turkey (2016–2019), Poland (2018), and more recently from Italy, Czech Republic, India (2019) and the US (2022), and are associated to several clonal lineages (i. e., ST11, 15, 23, 35, 39, 43, 48, 147, 268, 307, 336, 377, 383, 395, 874, 2096) [109,141–144] (**Table S3**).

Compelling evidence suggests that plasmids exchanges between successful pathotypes of *K. pneumoniae* (i. e., hvKp and MDR-Kp) represent the main culprit driving the evolution of this species. In this context, the increasingly reported emergence of p$^{MDR:hv}$ plasmids encoding KPC- or NDM-type carbapenemases and virulence-associated factors raises relevant clinical implications [79]. Although the carriage of genetic biomarkers strongly associated with hvKp is not a perfect surrogate for the hypervirulent phenotype *in vivo*, it should be noted that the acquisition of multiple siderophore systems has been recently shown to have a significant impact on cefiderocol, a recently approved siderophore cephalosporin with a potent anti-CRE activity [145,146]. As such, a lower activity of cefiderocol could be likely observed in these hvKp or emerging MDR-Kp^{hv} lineages because of a decreased drug uptake [147], further narrowing the available treatment options against CRE.

Of note, recent studies showed that both carbapenemase-encoding plasmids (i. e., carrying *bla*$_{KPC}$ or *bla*$_{NDM}$) or virulence plasmids can be transferred among these *K. pneumoniae* pathotypes via formation of outer membrane vesicles (OMVs) [148,149], spherical bilayer liposomes originating from the cell envelope of Gram-negatives [150], further advancing the knowledge about the molecular crosstalk between MDR-Kp and hvKp. Thus, as part of the bacterial secretome, OMVs represent a novel mechanism of horizontal gene transfer (i. e., in addition to plasmid conjugation), providing plasmids with an additional path for their dissemination and evolution.

It is clear that the epidemiology of MDR-Kp and hvKp is becoming more complex, with increasingly blurred limits between these pathotypes. Infection control practitioners and clinicians need nonetheless clinical microbiologists to rapidly identify and characterize these strains to promptly minimize their spread in the clinical setting. However, although several features of hvKp have been framed to date, there is no single genotypic of phenotypic biomarker that can define alone the hypervirulence phenotype, which is most likely the result of a complex interplay of multiple factors (e. g., capsular types, siderophores, hypermucoviscosity) [151]. This notion is largely supported by studies indicating that not all strains producing a K1 or K2-type capsule, or a defined subset of siderophores, are hvKp [10]; likewise, hypermucoviscosity should not be considered pathognomonic for hvKp (i. e., rather, just suggestive of hvKp), since this phenotype can be also exhibited by MDR-Kp [10].

7. Gut microbial communities as hot-spots for transmission and persistence of resistance plasmids

It is well known that both the patient-to-patient dissemination of successful HiRiCs and the horizontal transfer of carbapenemase-encoding plasmids between enterics find their place among the major trajectories shaping the epidemiology of CR-Kp (and more broadly of CRE) in the clinical setting. Although the relative contribution of each path remains difficult to establish, evidence for a pervasive within-patient transmission of carbapenemase-encoding plasmids from CR-Kp has recently been provided [152]. Horizontal gene transfer mediated by conjugative plasmids (as well as by other MGE) is regarded as a major driver of bacterial evolution and diversification, since plasmids' sharing is unlikely to occur solely in isolated single-species populations. This translates into the spread of conjugative carbapenemase-encoding plasmids from CR-Kp to other resident members of the gut microbiota (e. g., *Escherichia coli*, *C. freundii*, *Enterobacter cloacae*), once patients become colonized. Such trafficking provides plasmids with an increased chance to stably persist within the gut microbiota, and the bacterial hosts with an increased opportunity of accumulating even more resistance.

8. Conclusions

Over the past decades, CR-Kp emerged as a major public health threat, and its global dissemination largely contributed to the escalating burden of CRE [12,119]. This landscape was largely contributed by the notable ability of *K. pneumoniae* in acquiring, maintaining, and disseminating plasmids, which provided efficient platforms for the maintenance and dispersion of clinically relevant resistance determinants, including carbapenemases [51,127]. Indeed, carbapenemase-encoding plasmids played a pivotal role in evolution of carbapenem resistance in *K. pneumoniae*.

Modern advances in sequencing technologies have significantly improved our ability to decipher the huge diversity of plasmids involved in dissemination of carbapenem resistance determinants, and factors underlying their epidemiological success. The remarkable plasticity of plasmids, together with the genetic background provided by some successful epidemic clones *of K. pneumoniae*, overall resulted

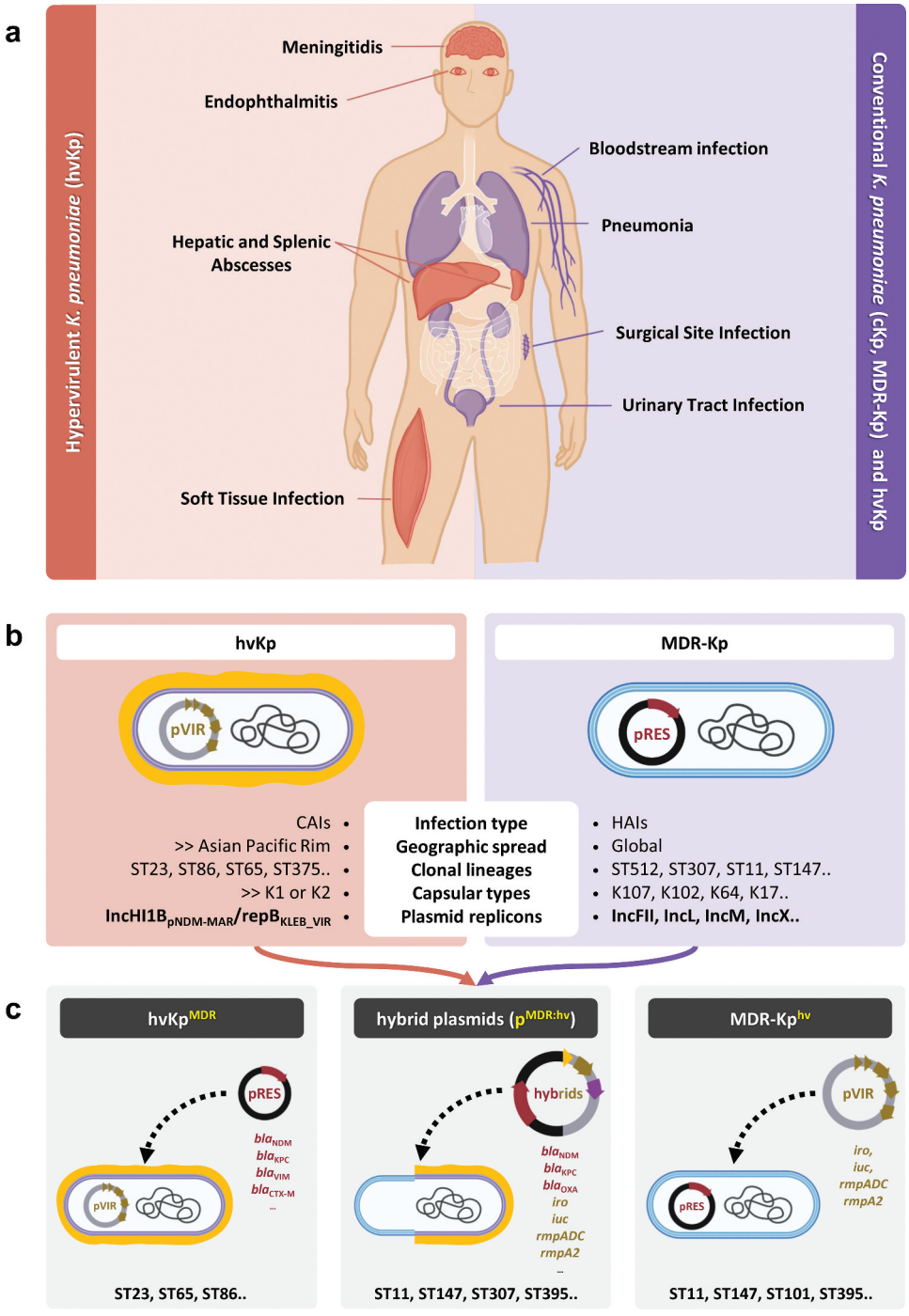

Figure 4. Overview of clinical and microbiological features of hypervirulent and classic (multidrug resistant) *K. pneumoniae* pathotypes and possible convergent evolutionary pathways. **Panel A.** Anatomical sites of documented infections by hypervirulent *K. pneumoniae* (hvKp) and classic *K. pneumoniae* (cKp) exhibiting a multidrug resistant phenotype (MDR-Kp); the panel was partially adapted from Gonzalez-Ferrer S. et al. [122]. **Panel B.** Epidemiological and genotypic features most frequently associated with the hvKp and MDR-Kp pathotypes. **Panel C.** Documented evolutionary paths involved in genetic convergence of resistance and virulence traits in *K. pneumoniae*.

in multiple dissemination pathways of carbapenemase genes. In fact, while some enzymes (e. g., OXA-48) have spread primarily via a single epidemic plasmid, which rapidly emerged in the clinical setting owing to its enhanced transfer abilities and pervasive diffusion among several HiRiCs [32,153,154], other carbapenemases (e. g., VIM and NDM) have spread via promiscuous associations of many diverse plasmids with diverse clonal lineages [32,127,155,156], with histories of variable success. Conversely, the stable association and coevolution of a single plasmid element with a given clonal lineage has been regarded as a key factor driving the global emergence and dissemination of other enzymes (e. g., KPC) [157].

The fast plasmid evolution through mutation and recombination events, as well as by gain or loss of MGE associated with resistance determinants, was another key factor

contributing to the success of these elements [158,159]. Indeed, owing to their high genetic plasticity, plasmids can mediate both accretion and modulation of antimicrobial resistance, resulting in drastic phenotypic changes. In that regard, the recent description of CR-Kp strains producing novel KPC variants (e. g., KPC-31) associated with reduced susceptibility to CZA and cefiderocol, and of strains exhibiting an increased bla_{KPC} gene dosage mediating cross-resistance to novel BLICs (i. e., CZA, MVB, IMR), are paradigmatic examples of these abilities [23,103,160,161].

The observation that carbapenem resistance in *K. pneumoniae* is mainly mediated by genes located on plasmids poses also relevant hurdles from an infection prevention and control point of view. Indeed, plasmids can often disseminate intra- and inter-species, thus providing a gene pool easily accessible to bacteria belonging to different taxonomic groups and ecosystems [162], possibly causing 'plasmid-mediated gene epidemics' [51]. Indeed, in some cases (e. g., for OXA-48 and KPC), plasmid-encoded carbapenemases could be silently disseminated in bacteria, considering that the presence of a given carbapenemase gene could lead to higher carbapenem MICs that are still lower than resistance breakpoints [163,164]. Moreover, the use of automated systems for antimicrobial susceptibility testing may not efficiently detect all carbapenemase producers [165], hampering the possibility of implementing more powerful tools for epidemiological surveillance such as those based on genome sequencing.

Taken together, these findings point out that plasmids can behave as highly plastic genetic toolkits, providing formidable evolutionary opportunities to *K. pneumoniae*, likely explaining its successful emergence and persistence in clinical settings.

9. Expert opinion

K. pneumoniae has been well established in the hospital environment and is a leading cause of HCAIs. Although naturally susceptible to several antibiotics, *K. pneumoniae* is a notorious 'collector' of MDR plasmids, including those carrying carbapenemase genes. As a consequence, we have witnessed a global crisis of unprecedented dimensions due to the rapid dissemination of carbapenem resistance among *K. pneumoniae* [15]. Indeed, CP-Kp have been identified worldwide and are considered one of the most important clinical and public health issues, contributing to one of the modern epidemics.

The worldwide spread of CR-Kp strains can be viewed as an ecological consequence of the interaction between the overuse of antimicrobial agents in healthcare environments and the underlying biological machinery governing the emergence, establishment, and dissemination of carbapenemase-encoding determinants in the *K. pneumoniae* population.

As it has been discussed here, two are the key players for the spreading of carbapenemases and the shaping of CP-Kp population, namely successful bacterial clones and, most importantly, plasmids, and associated MGEs [19,51,52,57,58]. This complex epidemiological picture is based on the emergence and establishment of CP-Kp HiRiCs in different clinical settings and the existence of a huge variability of carbapenemase-encoding plasmids, including different replicons, multireplicons, chimeric plasmids, and genetic platforms carrying carbapenemase genes, that can be transferred to other bacterial species. In CP-Kp clones, carbapenemase genes are almost invariably associated to a wide repertoire of other antibiotic resistance genes carried either by the same or different plasmids. These arrangements ultimately led to the emergence of complex MDR phenotypes, including resistance to aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, chloramphenicol, and tetracyclines [52,58]. Horizontal gene transfer plays a fundamental role in the continuously changing pattern of CP-Kp resistomes, dynamically shaping their MDR phenotypes. Moreover, the convergence of carbapenemase-encoding and virulence plasmids has led to the emergence of novel *K. pneumoniae* pathotypes, as hvKp clones that are also resistant to carbapenems [10,122]. To that end, plasmids and associated mobile structures are important contributors for the evolution and diversification of CP-Kp population, through acquisition of novel genetic elements, genes, or new mutations. The result of this reshuffling, which cannot be predicted, has a huge impact on the available treatment options for CP-Kp, which can become very limited. Different scenarios have been proposed based on different plasmid/lineages combinations (e. g., one plasmid/multiple lineages as for bla_{OXA-48}-like, multiple plasmids/multiple lineages as for bla_{VIM} and bla_{NDM}, and multiple plasmids/one lineage for bla_{KPC}), which should be taken into consideration for the investigation of carbapenemase producers by surveillance systems and for the design of new infection control interventions [32].

The large body of research on CP-Kp (a) elucidated the scenarios underlying transmission of these strains at local, national, and international level, which are clonal or in other cases plasmid epidemics [32]; (b) deciphered the resistome and the genetic features of circulating carbapenemase-encoding plasmids [51,52,58]; (c) allowed the designing of novel diagnostic methodologies for the reliable identification of carbapenemases [19,116]; (d) led to the development of epidemiological tools for surveillance purposes; and (e) provided new insights that can be used for the development of novel therapeutic approaches targeting CP-Kp infections [19,116].

On the other hand, the observed phenotypic and genetic variability of carbapenemase producers and the respective vehicles/plasmids poses two main drawbacks. Firstly, in some cases, carbapenemases conferring unusual resistance profiles are not correctly identified by existing diagnostic tests. Secondly, the implementation of rational infection control policies is hampered by the rapid adaptation potential of CP-Kp strains and by the emergence of novel carbapenemases of diverse origin that can evade their detection by a targeted active surveillance system. Owing to the plasticity and evolutionary potential of carbapanemase-encoding plasmids, it should be pointed out that continuous study of the biology of resistance plasmids is imperative for designing rational policies to control their dissemination, as well as to find novel treatments for CP-Kp infections. In addition, CP-Kp strains can serve as a pool of carbapenemase gene donors to other Enterobacterales, further pointing out the importance to limit the spread of such strains in the clinical setting as soon and as effectively as possible.

In the near future, integrated approaches for the study of CP-Kp infections are needed in order to translate basic knowledge into clinical interventions. As the epidemic progresses, the aim should be i) to accumulate additional genomic data for carbapenemase producers, which have been reported to evolve rapidly, ii) to develop new rapid diagnostic tools, enabling detection of emerging resistance mechanisms (e. g., carbapenemase genes copy number variations, enzymatic variants posing challenges for laboratory testing, mutations conferring major phenotypic changes in antibiotic susceptibility) and iii) based on the above, to improve monitoring systems allowing a fast, real-time, recognition of known or emerging threats. Moreover, the development of Clinical Decision Supporting Systems based on clinical, epidemiological, microbiological, and genomic data would assist therapeutic decisions and would lead to the optimization of therapeutic protocols. The stakeholders for these efforts are academics, researchers, Public Health Authorities, and the pharmaceutical industry.

Funding

This work was partially supported by a research grant (RF-2016-02364584) by the Italian Ministry of Health to GMR and by the research grant financed by the European Union – Next Generation EU - PRIN 2022 (20224T3X8K) by the Italian Ministry of Education, University and Research to MMD.

Declaration of interest

Outside the submitted work, VDP reports speakers' bureaus from Ada. Outside the submitted work, GMR reports grants or contracts from Ada, Alifax, Angelini, Arrow Diagnostics, Biomedical Service, bioMérieux, Cepheid, Hain Lifescience GmbH, Menarini, Meridian, MSD, Nordic Pharma, Qlinea, Quantamatrix, Quidel, Qvella, SD Biosensor, Seegene, Shionogi, Symcel; consulting fees from bioMérieux, MSD; payment or honoraria for lectures, presentations, speakers' bureaus, manuscript writing or educational events by bioMérieux, Menarini, MSD, Pfizer, Shionogi, Relab. Outside the submitted work, MMD reports contracts from Glaxo-Smith Kline and payments for software consultancy and congresses attendance by Arrow Diagnostics. Outside the submitted work, SP reports contracts from Molteni Therapeutics and congresses attendance by Arrow Diagnostics.

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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