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Genetic plurality of bla_{KPC-2}-harboring plasmids in high-risk clones of *Klebsiella pneumoniae* of environmental origin

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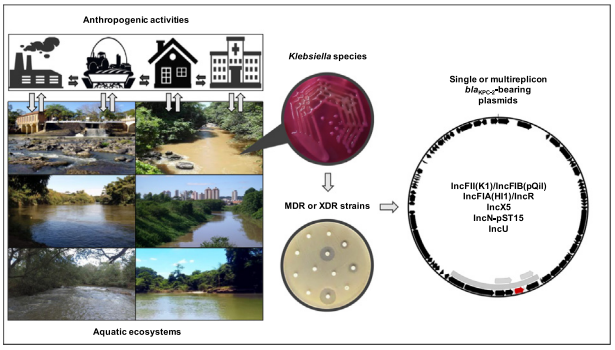
HIGHLIGHTS

- Dispersion of XDR or MDR high-risk clones was observed in aquatic ecosystems.
- The coexistence of bla_{KPC-2} and *mer* operon (mercury tolerance) was found.
- Diversity of single and multireplicon plasmids bearing the bla_{KPC-2} gene was identified.
- Comparative analysis revealed interspecies, intraspecies, and clonal transmission.
- The long persistence of plasmids at the human-animal-environmental interface is discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

International high-risk clones of *Klebsiella pneumoniae* are important human pathogens that are spreading to the environment. In the COVID-19 pandemic scenario, the frequency of carbapenemase-producing strains increased, which can contribute to the contamination of the environment, impacting the surrounding and associated ecosystems. In this regard, KPC-producing strains were recovered from aquatic ecosystems located in commercial, industrial, or agricultural areas and were submitted to whole-genome characterization. *K. pneumoniae* and *Klebsiella quasipneumoniae* subsp. *quasipneumoniae* strains were assigned to high-risk clones (ST11, ST340, ST307) and the new ST6325. Virulome analysis showed genes related to putative hypervirulence. Strains were resistant to almost all antimicrobials tested, being classified as extensively drug-resistant or multidrug-resistant. In this context, a broad resistome (clinically important antimicrobials and hazardous metal) was detected. Single replicon (IncX5, IncN-pST15, IncU) and multireplicon [IncFII(K1)/IncFIB(pQil), IncFIA(HI1)/IncR] plasmids were identified carrying the bla_{KPC-2} gene with Tn*4401* and non-Tn*4401* elements. An unusual association of bla_{KPC-2} and *qnrVC1* and the coexistence of bla_{KPC-2} and *mer* operon (mercury tolerance) was found. Comparative analysis revealed that bla_{KPC-2}-bearing plasmids were most similar to plasmids from *Enterobacterales* of Brazil, China, and the United States, evidencing the long persistence of plasmids at the human-animal-environmental interface. Furthermore, the presence of uncommon plasmids, displaying the interspecies, intraspecies, and clonal transmission, was highlighted. These findings alert for the spread of high-risk clones producing bla_{KPC-2} in the environmental sector and call attention to rapid dispersion in a post-pandemic world.

1. Introduction

Carbapenem-resistant *Enterobacteriaceae* are critical-priority bacteria, making a threat primarily in hospitals (Tacconelli et al., 2018). The

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carbapenems are considered agents of last resort for severe bacterial infections, but now are threatened by widespread resistance caused mainly by plasmid-encoded carbapenemases (García-Betancur et al., 2021). In addition to carbapenem resistance, carbapenemase-producing strains often also exhibit resistance to other clinically important antimicrobials, highlighting polymyxins (Potter et al., 2016; Bonomo et al., 2018). In the environment, carbapenemase-positive strains have emerged, implying a risk of transmission for humans and animals in clinical and non-clinical settings, and more recently for fresh vegetables (Brilhante et al., 2021; Cherak et al., 2021; Lopes et al., 2022).

Klebsiella pneumoniae Carbapenemase (KPC), encoded by bla_{KPC} gene, is one of the most common carbapenemases worldwide and, to date (January 2023), there are >140 variants with the bla_{KPC-2} gene being the most reported (Munoz-Price et al., 2013; Naas et al., 2017). The transfer of carbapenemases is facilitated by mobile genetic elements (MGEs) and the bla_{KPC-2} gene with Tn*4401* and non-Tn*4401* elements (NTE_{KPC}) have been identified in different groups of plasmids (Brandt et al., 2019). In addition, high-risk clones of *Klebsiella pneumoniae*, especially those from clonal groups (CG) 258 and 307, contain a diverse repertoire of MGEs, contributing to the emergence of antimicrobial resistance (Shropshire et al., 2022).

Aquatic environments are vulnerable to the discharge of wastes, and hospital effluents strongly contribute to the occurrence of carbapenemases in this sector (Cahill et al., 2019). From a One Health perspective, the environment contributes to the expansion of antimicrobial resistance genes (ARGs) and their dissemination for different areas; however, in-depth genomic analysis of the horizontal transfer of plasmids harboring carbapenemases to the environment remains limited. Therefore, this study aimed to perform a whole-genome sequence-based analysis of bla_{KPC-2}-positive *Klebsiella* species from surface waters of Brazil.

2. Materials and methods

2.1. Sampling and bacterial strains

In February 2020, 44 sampling sites of rivers and streams located on São Paulo State, Brazil, were investigated for the presence of carbapenem-resistant *Klebsiella* species. One liter of each water sample was collected, transported, and processed by the membrane filtration technique (Forster and Pinedo, 2015) using cellulose nitrate filters with pore sizes of 0.45 μm (Sartorius, Germany). Subsequently, the filters were placed on plates of MacConkey agar (Oxoid, UK) supplemented with 4 mg/L of meropenem, which were incubated at 35 ± 2 °C for up to 48 h. Finally, random mucoid and lactose fermenting colonies were selected, subcultured, and stocked. Genomic DNA was extracted using the PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA). Molecular identification was performed by conventional polymerase chain reactions using species-specific genes (Fonseca et al., 2017).

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility was first determined by disk diffusion method to amoxicillin/clavulanate, piperacillin/tazobactam, cefazolin, cefoxitin, ceftazidime, ceftazidime/avibactam, ceftriaxone, ceftaroline, cefepime, aztreonam, imipenem, meropenem, nalidixic acid, ciprofloxacin, gentamicin, amikacin, tetracycline, minocycline, sulfamethoxazole/trimethoprim, chloramphenicol, and nitrofurantoin. Minimum inhibitory concentrations (MICs) for most of the above-mentioned antimicrobials with the addition of colistin and tigecycline were assessed by broth microdilution or *E*-test® methods. All results were interpreted according to Clinical and Laboratory Standards Institute breakpoints (CLSI, M100, 30th, 2020), except for tigecycline, for which it was used the Brazilian Committee on Antimicrobial Susceptibility Testing/European Committee on Antimicrobial Susceptibility Testing breakpoints (BrCast/EUCAST, v.11.0, 2021). Strains were classified as multidrug-resistant (MDR) or extensively drug-resistant (XDR) according to Magiorakos et al. (2012).

2.3. Identification of carbapenemases

Phenotypic detection of carbapenemases was performed by Carbapenemase Detection Kit (Cecon, Brazil). Carbapenemase-encoding genes were screened using conventional polymerase chain reactions (PCR) and confirmed by Sanger sequencing (Pitout et al., 2005; Dallenne et al., 2010; Peirano et al., 2011).

2.4. Whole-genome sequencing and analysis

Whole-genome sequencing was performed using the Illumina MiSeq platform (Illumina Inc., USA). The reads trimming and filtering were carried out using BBDuk v.38.84 embedded in Geneious Prime® v.2022.2.2 (Biomatters Ltd., New Zealand). The genome was *de novo* assembled by SPAdes v.3.15.2 (Bankevich et al., 2012) and annotated using the RAST server (https://rast.nmpdr.org/rast.cgi). Resistome and plasmid replicons were identified using ResFinder v.4.1 and PlasmidFinder v.2.1, respectively, available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). Neutral or deleterious mutations in targets related to colistin resistance were determined in-house by Geneious Prime® v.2022.2.2 and PROVEAN (http://provean.jcvi.org/index.php) using *K. pneumoniae* MGH 78578 (GenBank accession number CP000647) as reference.

Virulome, multilocus sequence typing, serotyping, and metal tolerance genes were determined by Kleborate (https://github.com/katholt/Kleborate) and BIGSdb-Pasteur (https://bigsdb.pasteur.fr/klebsiella/). Plasmid contigs were predicted using mlplasmids v.2.1.0 (https://sarredondo.shinyapps.io/mlplasmids/). The plasmid assembly was carried out using the hybrid strategy described by Cerdeira et al. (2011). Insertion sequences were analyzed using ISfinder (https://www-is.biotoul.fr/index.php). Circular and linear comparisons of plasmid sequences were performed using BRIG (https://brig.sourceforge.net/) and Geneious Prime® v.2022.2.2.

2.5. Plasmid transfer and stability

Plasmid transfer was performed by conjugation using *Escherichia coli* J53 resistant to sodium azide in MacConkey agar (Oxoid, United Kingdom) supplemented with sodium azide (200 mg/L) and meropenem (2 mg/L), and transconjugants were confirmed by PCR for the detection of bla_{KPC} gene. Plasmid stability was evaluated by daily serial passages of strains on Mueller Hinton agar (Kasvi, Spain) without antimicrobial at 35 ± 2 °C (De Gelder et al., 2007). Then, random colonies were tested for the presence of the bla_{KPC} gene.

3. Results

3.1. Sampling sites and KPC-producing strains

Strains harboring the bla_{KPC} gene (EW666, EW775, EW671, EW608, EW606) were found in 9 % of the sampling sites studied (Supplementary Fig. S1) and were submitted to whole-genome characterization (Supplementary Table S1). The aquatic ecosystems (rivers and streams) where the bla_{KPC}-producing strains were recovered are located in commercial, industrial, or agricultural areas and there are records of high *E. coli* CFU counts in these aquatic ecosystems (up to 300,000 CFU/100 mL) according to the annual reports of CETESB (https://cetesb.sp.gov.br/aguas-interiores/publicacoes-e-relatorios/), suggesting the influence of anthropogenic activities in water contamination. Strains EW666, EW775, EW671, and EW608 were identified as *K. pneumoniae*, while EW606 strain was recognized as *K. quasipneumoniae* subsp. *quasipneumoniae*. Besides, strains EW608 and EW671 showed the mucoviscous phenotype, while EW666 strain presented the hypermucoviscous phenotype.

3.2. Antimicrobial resistance phenotype

In general, bla_{KPC}-producing strains were MDR or XDR since showed resistance to agents of the following antimicrobial classes: β-lactams,

Table 1
Genetic characteristics of *bla*$_{KPC-2}$-producing strains.

Strain	MLST[a]	Serotyping	Virulence determinants	Antimicrobial resistance genes	*bla*$_{KPC-2}$ localization	Other plasmid replicons
EW666	ST11	O1/O2v1: KL64	*entB*, *iutA*, *fyuA*, *irp1*, *irp2*, *ybtA*, *ybtE*, *ybtP*, *ybtQ*, *ybtS*, *ybtT*, *ybtU*, *ybtX*, *mrkA*, *mrkB*, *mrkC*, *mrkC*, *mrkD*, *mrkF*, *mrkH*, *mrkI*, *mrkJ*, *clbA*, *clbB*, *clbC*, *clbD*, *clbE*, *clbF*, *clbG*, *clbH*, *clbI*, *clbL*, *clbM*, *clbN*, *clbO*, *clbP*, *clbQ*, *clbR*	*bla*$_{KPC-2}$, *bla*$_{OXA-2}$, *bla*$_{OXA-9}$, *bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *oqxA*, *oqxB*, *aac(6′)-Ib3*, *aac (3)-IIa*, *sul1*, *fosA*	IncFII(K1)/IncFIB (pQil)	IncFIB(K), Col440I
EW775	ST340	O4:KL151	*entB*, *iutA*, *mrkA*, *mrkB*, *mrkC*, *mrkD*, *mrkF*, *mrkH*, *mrkI*, *mrkJ*	*bla*$_{KPC-2}$, *bla*$_{CTX-M-15}$, *bla*$_{SHV-11}$, *qnrB1*, *oqxA*, *oqxB*, *aac(3)-IId*, *dfrA14*, *mph(A)*, *catA2*, *fosA*	IncFIA(HI1)/IncR	IncFII(K), IncFIB(K), Col440I
EW671	ST307	O1/O2v2: KL102	*entB*, *iutA*, *mrkA*, *mrkB*, *mrkC*, *mrkC*, *mrkD*, *mrkF*, *mrkH*, *mrkI*, *mrkJ*	*bla*$_{KPC-2}$, *bla*$_{CTX-M-15}$, *bla*$_{TEM-1B}$, *bla*$_{SHV-28}$, *oqxA*, *oqxB*, *aac(3)-IV*, *aph(3″)-Ib*, *aph(4)-Ia*, *aph(6)-Id*, *aadA1*, *aadA2b*, *tet(A)*, *sul2*, *sul3*, *dfrA14*, *cmlA1*, *fosA*	IncX5	IncFII(K), IncFIB(K), IncR, Col440I, Col440II, ColpVC, Col(pHAD28)
EW608	ST11	O1/O2v1: KL64	*entB*, *iutA*, *fyuA*, *irp1*, *irp2*, *ybtA*, *ybtE*, *ybtP*, *ybtQ*, *ybtS*, *ybtT*, *ybtU*, *ybtX*, *mrkA*, *mrkB*, *mrkC*, *mrkD*, *mrkF*, *mrkH*, *mrkI*, *mrkJ*, *clbA*, *clbB*, *clbC*, *clbD*, *clbE*, *clbF*, *clbG*, *clbH*, *clbL*, *clbM*, *clbN*, *clbP*, *clbQ*, *clbR*	*bla*$_{KPC-2}$, *bla*$_{CTX-M-2}$, *bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *oqxA*, *oqxB*, *aac(6′)-Ib3*, *aac(3)-IIa*, *sul1*, *dfrA30*, *catA1*, *fosA*	IncN-pST15	IncFII(K), IncFIB(K), IncFIB(pQil), ColRNAI
EW606	ST6325	O3/O3a: KL58	*entB*, *kfuA*, *kfuB*, *kfuC*, *mrkB*, *mrkD*, *mrkF*, *mrkI*, *mrkJ*	*bla*$_{KPC-2}$, *bla*$_{OKP-A-5}$, *qnrVC1*, *oqxA*, *oqxB*, *aac (6′)-Ib3*, *aadA1*, *fosA*	IncU	IncFII(K), IncFIB(K), IncFIB (pQil), IncR, Col440I

[a] Multilocus sequence typing, MLST; Sequence type, ST.

polymyxins, aminoglycosides, fluoroquinolones, folate pathway antagonists, tetracyclines, phenicols, and nitrofurans. In this regard, high MICs to clinically important antimicrobial agents (amoxicillin/clavulanate, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, gentamicin, tetracycline, chloramphenicol, and colistin) were observed mainly in XDR strains. On the other hand, all strains were susceptible to ceftazidime/avibactam and most were susceptible to tigecycline, except the EW666 strain that was resistant (MIC 4 mg/L) (Supplementary Tables S2 and S3).

3.3. Molecular typing and serotyping

Strains were assigned to STs (ST11 O1/O2v1:KL64, ST340 O4:KL151, ST307 O1/O2v2:KL102 *K. pneumoniae* and ST6325 O3/O3a:KL58 *K. quasipneumoniae*). Amongst these, the new ST6325 was the single locus variant (new 336 allele of *gapA*) of ST144. Of highlight, *K. pneumoniae* strains belonged to the high-risk clones of CG258 and CG307. Curiously, strains EW608 and EW666 were isolated from different aquatic ecosystems and presented the same ST11 O1/O2v1:KL64, while strains EW608 and EW606 were obtained from the same sampling site and presented different species, STs, and serotypes (Table 1).

3.4. Resistome

In addition to the *bla*$_{KPC-2}$ gene, strains also carried intrinsic and acquired resistance genes to β-lactams (*bla*$_{CTX-M-2}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-2}$, *bla*$_{OXA-9}$, *bla*$_{TEM-1B}$, *bla*$_{SHV-11}$, *bla*$_{SHV-28}$, and *bla*$_{OKP-A-5}$), aminoglycosides [*aadA1*, *aadA2b*, *aac(3)-IIa*, *aac(3)-IId*, *aac(6′)-Ib3*, *aac(3)-IV*, *aph(3″)-Ib*,

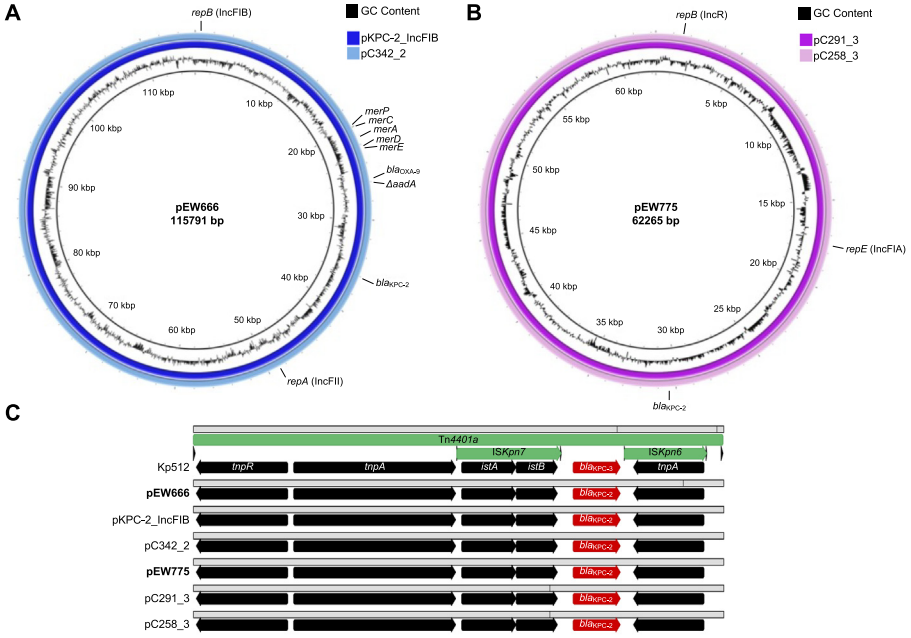

Fig. 1. Multireplicon plasmids harboring the *bla*$_{KPC-2}$ gene with Tn*4401a* isoform. A) Comparison of the IncFII(K1)/IncFIB(pQil) plasmid from this study (in bold) (pEW666, GenBank accession number JAQIIP010000001) with others from humans in China (pKPC-2_IncFIB, GenBank accession number CP050168), and the United States (pC342_2, GenBank accession number CP067726). B) Comparison of the IncFIA(HI1)/IncR plasmid from this study (in bold) (pEW775, GenBank accession number JAQIIQ010000001) with others from humans in the United States (pC291_3, GenBank accession number CP067811; pC258_3, GenBank accession number CP067888). The colored rings of figs. A and B denote similarity between the plasmid sequences. C) Alignment of Tn*4401a* isoform found in the beforementioned plasmids with reference Kp512 strain (GenBank accession number KT378596). The gray lines represent shared regions of homology.

aph(4)-Ia, and *aph(6)-Id*], fluoroquinolones (*qnrB1*, *qnrVC1*, *oqxA*, and *oqxB*), folate pathway antagonists (*sul1*, *sul2*, and *sul3*), trimethoprim (*dfrA14* and *dfrA30*), tetracyclines [*tet(A)*], phenicols (*cmlA1*, *catA1*, and *catA2*), fosfomycins (*fosA*), and macrolides [*mph(A)*] (Table 1). Known mutations in OmpK37, OmpK36, and AcrR/GyrA/ParC with a potential contribution to increased resistance for carbapenems, cephalosporins, and fluoroquinolones, respectively, were detected. In addition, deleterious mutations in MgrB (Q30*), PhoQ (W211R), and PmrB (R256G) were identified and may be related to colistin resistance (Supplementary Table S4). Furthermore, tolerance genes to silver (*silESRCFBAGP*), copper (*pcoABCDRSE*), arsenic (*arsRABCD*), and mercury (*merRTPCADE*) were detected in *K. pneumoniae* strains.

3.5. Virulence genotyping

Overall, genes related to siderophores, exotoxin, and biofilm formation were detected, which encoded enterobactin (*entB*), yersiniabactin (*ybt*), aerobactin (*iutA*), iron uptake (*kfu*), colibactin (*clb*), and type 3 fimbriae (*mrk*) (Table 1). In addition to putative hypervirulence, strains EW666 and EW608 carried the ICE*Kp*10 structure.

3.6. Plasmid-mediated bla_{KPC-2} gene

Interestingly, the bla_{KPC-2} gene with different genetic contexts was located on distinct plasmids (Table 1). In general, bla_{KPC-2}-mediated plasmids harboring genes related to replication, partition, conjugation, maintenance, stability, and metal tolerance. Strain EW666 harbored a plasmid, named pEW666, co-carrying bla_{KPC-2}, bla_{OXA-9}, Δ*aadA* (111 bp), and *mer* genes. The pEW666 plasmid belonged to the IncFII(K1)/IncFIB(pQil) and was 115,791 bp in length, containing 54 % GC content. BLASTn analysis revealed that pEW666 was most related (100 % query coverage and 99.8 % nucleotide identity) to plasmids from *K. pneumoniae* strains of humans in China and the United States (Fig. 1A). Strain EW775 carried a 62,265 bp-long multireplicon IncFIA(HI1)/IncR plasmid, named pEW775, with 53.4 % GC content. Comparative analysis showed that pEW775 was similar (100 % query coverage and 99.6 % nucleotide identity) only with plasmids from two clinical *K. pneumoniae* strains of the United States (Fig. 1B). The bla_{KPC-2} gene with Tn*4401a* isoform was identified in pEW666, pEW775, and their closely related plasmids (Fig. 1C).

Strain EW671 carried an IncX5 plasmid, named pEW671, with 41,872 bp in length and 45.7 % GC content. This plasmid was related (> 98 % query

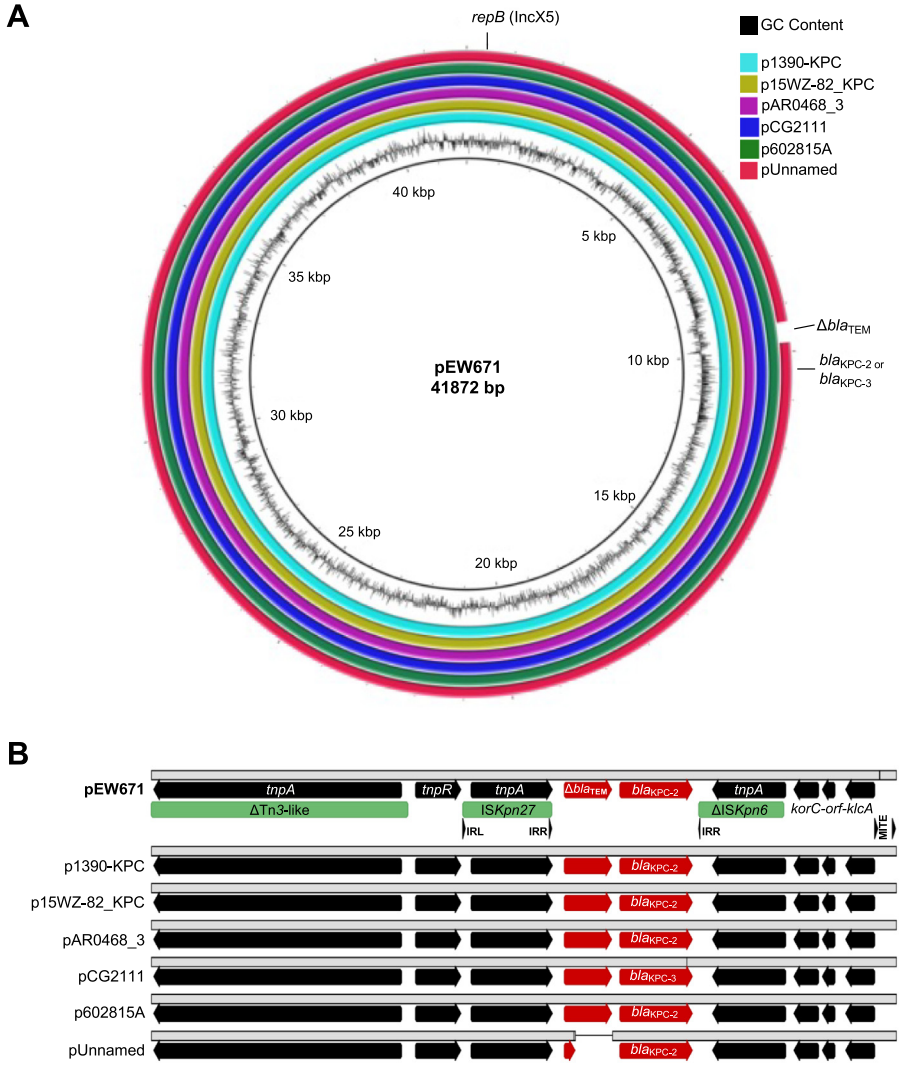

Fig. 2. IncX5 plasmids carrying the bla_{KPC-2} gene with a Tn*3*-family unit transposon. A) Comparison of the IncX5 plasmid from this study (in bold) (pEW671, GenBank accession number JAQIIR010000001) with others from *K. pneumoniae* (pP1390-KPC, GenBank accession number MF344555), *K. variicola* (p15WZ-82_KPC, GenBank accession number CP032355), *E. asburiae* (pAR0468_3, GenBank accession number CP083837), *S. marcescens* (pCG2111, GenBank accession number CP081510), *R. ornithinolytica* (p602815A, GenBank accession number MZ753458), and *E. coli* (pUnnamed, GenBank accession number KY062156) of humans in China. The colored rings of denote similarity between the plasmid sequences. B) Alignment of genetic environment of bla_{KPC-2} in the beforementioned plasmids. IRL, left inverted repeat. IRR, right inverted repeat. MITE, miniature inverted-repeat transposable element. The gray lines represent shared regions of homology.

coverage and 99.9 % nucleotide identity) to plasmids harboring bla_{KPC-2} or bla_{KPC-3} genes from *Enterobacterales* (*K. pneumoniae*, *Klebsiella variicola*, *Enterobacter asburiae*, *Serratia marcescens*, *Raoultella ornithinolytica*, and *Escherichia coli*) of humans in China (Fig. 2A). The region harboring the bla_{KPC} gene contained a Tn*3*-family unit transposon with the core sequence composed by IS*Kpn27*-Δbla_{TEM}-bla_{KPC}-ΔIS*Kpn6* linked to the gene cluster *korC-orf-klcA* (Fig. 2B).

An IncN-pST15 plasmid, name pEW608, was identified in EW608 strain. The pEW608 plasmid was 53,267 bp in length, containing 52.8 % GC content. Comparative analysis showed that pEW608 plasmid shared high nucleotide identity with various plasmids distributed worldwide. Furthermore, the pEW608 plasmid showed >99.8 % nucleotide identity with plasmids previously identified between 2009 and 2019 in strains of *K. pneumoniae* and *E. coli* at the human-animal-environment interface of southeastern Brazil (Fig. 3A). The bla_{KPC-2} gene with Tn*4401b* isoform was identified in these Brazilian plasmids (Fig. 3B).

A fragment of IncU plasmid (16,160 bp) co-harboring bla_{KPC-2}, Δbla_{TEM} (189 bp), *qnrVC1*, and Δ*dfrA31* (138 bp) was identified in EW606 strain. The genetic context of bla_{KPC-2} and *qnrVC1* were ΔIS*Kpn6*-bla_{KPC-2}-Δbla_{TEM}-ΔTn*3*-like and Δ*dfrA31*-*qnrVC1*-IS*21*-like, respectively, and both shared high nucleotide identity (>99 %) with others available at public databases. Strikingly, IncU fragment was similar only to a recently described plasmids from *Enterobacter kobei* strains of coastal waters in Brazil (Fig. 4), evidencing an unusual association of bla_{KPC-2} and *qnrVC1* in a same environmental strain. Finally, the sequence length of complete plasmids supported the plasmid sizes visualized after plasmid extraction. Plasmids transfer by conjugation was successful for all plasmids, except IncU, and all strains maintained the bla_{KPC-2}-harboring plasmids for 30 days in antimicrobial-free culture medium.

4. Discussion

Carbapenemase-positive ST11, ST340, and ST307 are international high-risk clones linked to the epidemiological success of bla_{KPC} in nosocomial settings, whereas, in veterinary settings and in the environment, they are adapting and progressing, respectively (Wyres and Holt, 2018; Schmidt et al., 2020; Arcari and Carattoli, 2022). The presence of these clones in surface waters, especially those affected vigorously by pollutants, accelerates the transmission of pathogenic strains and antimicrobial resistance, implicating the One Biosecurity (Larsson and Flach, 2022; Hulme,

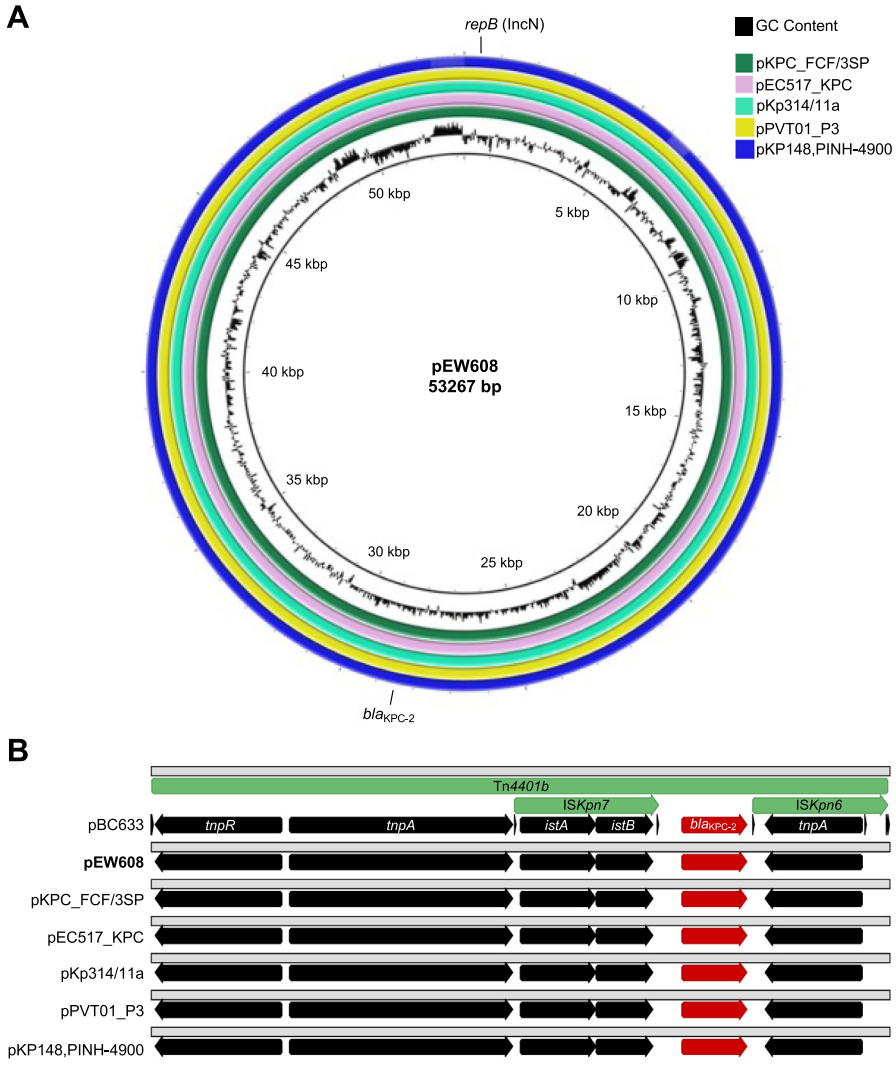

Fig. 3. IncN-pST15 plasmids harboring the bla_{KPC-2} gene with Tn*4401b* isoform. A) Comparison of the IncN-pST15 plasmid from this study (in bold) (pEW608, 2020, GenBank accession number JAQIIS010000001) with others from humans (pKPC_FCF/3SP, 2009, GenBank accession number CP004367; pEC517_KPC, 2011, GenBank accession number CP018963; pKp314/11a, 2011, GenBank accession number KX276209), animal (dog) (pPVT01_P3, 2019, GenBank accession number JABSUB010000003), and environmental (water) (pKP148,PINH-4900, 2011, GenBank accession number KX062091) in Brazil. The colored rings denote similarity between the plasmid sequences. B) Alignment of Tn*4401b* isoform found in the beforementioned plasmids with reference pBC633 plasmid (GenBank accession number EU176012). The gray lines represent shared regions of homology.

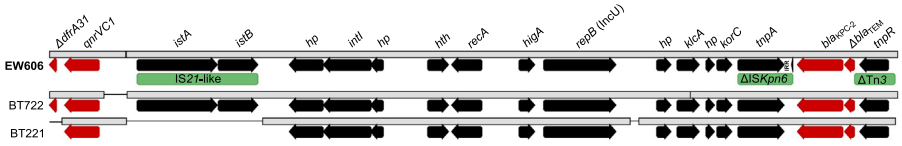

Fig. 4. Schematic representation of IncU fragments housing bla_{KPC-2} and *qnrVC1* genes in *K. quasipneumoniae* subsp. *quasipneumoniae* strain EW606 (this study; in bold; GenBank accession number JAQIIT010000074) and *E. kobei* strains BT722 (IncP6/U) (GenBank accession number JANINA010000003) and BT221 (IncU) (GenBank accession number JANINB010000003) from Brazilian coastal waters. hp, hypothetical protein. IRR, right inverted repeat. The gray lines represent shared regions of homology.

2020). The transfer of these pathogens to humans occurs mainly through contact and consumption of contaminated water and food, resulting in difficult treatment, longer hospital stays, increased healthcare costs, and higher mortality rates (Santos and Secoli, 2019; UNEP, 2023). Besides, resistance to colistin and even tigecycline was observed in bla_{KPC-2}-positive strains, which raises concerns and creates a therapeutic challenge, as these antimicrobial agents are important against infections caused by carbapenem-resistant *Enterobacteriaceae* (Van Duin et al., 2013; Ni et al., 2016).

Worryingly, the advance of the COVID-19 pandemic has increased the frequency of MDR and carbapenemase-producing strains, further accelerating the spread mainly of carbapenem resistance and posing another major challenge for One Health (Polly et al., 2022; Thomas et al., 2022). In addition, the coexistence of different carbapenemases is emerging in the Caribbean and is becoming frequent in Latin America (García-Betancur et al., 2021; PAHO, 2021). In this context, the prevalence of bla_{KPC} in the sampling sites studied may be underestimated since the samples were collected in the initial period of COVID-19 cases.

Xenogenetic pollutants do not respect geopolitical boundaries and find in aquatic ecosystems the ideal configurations to harbor clinically relevant ARGs (Gillings et al., 2018). The bla_{KPC} gene is typically plasmid-borne in *Enterobacterales* and various bla_{KPC}-containing mobile elements have already been reported, with Tn*4401*, a Tn*3*-based transposon, being the most common (Carattoli, 2013; Chen et al., 2014). In this study, the bla_{KPC} gene was embedded within Tn*4401a* and Tn*4401b* isoforms on IncF, IncR, and/or IncN plasmids, corroborating with genetic data about bla_{KPC}-producing strains belonging to the CG258 (Conlan et al., 2014; Stohr et al., 2021). Multireplicon plasmids composed mainly of IncF backbones have emerged as important carriers of bla_{KPC} and have a greater capacity to carry multiple ARGs when compared to single replicons, endorsing our findings (Feng et al., 2019; Piccirilli et al., 2021; Wang et al., 2021).

IncX plasmids, especially IncX5, harboring the bla_{KPC-2} are infrequently found. In the Americas, these plasmids have only been reported in *K. pneumoniae* strains from humans (Chen et al., 2013; Souza et al., 2019). The bla_{KPC-2} was carried by NTE_{KPC} in IncX5 plasmids that appears to be related to IncX6 and IncP plasmids, showing the evolution of bla_{KPC} mobilization (Yao et al., 2017; Ghiglione et al., 2021; Hala et al., 2019). On the other hand, IncN-pST15 plasmids can play an important role in the transmission of carbapenem resistance in *K. pneumoniae* lineages (Rada et al., 2020). In the last decade in Brazil, bla_{KPC-2}/IncN-pST15 was identified in non-CG258 (ST442, ST437) from human and environmental origins (Pérez-Chaparro et al., 2014; Oliveira et al., 2014), and more recently in CG258 (ST11) from a dog, evidencing the long persistence of these plasmids in different sources and supporting the IncN-pST15 as a One-Health plasmid (Sellera et al., 2021).

The first description of the coexistence of bla_{KPC-2} and *qnrVC* was in *Citrobacter freundii* of China (Zhu et al., 2020), while the two subsequent ones were in species of *Klebsiella* and *Enterobacter* from Brazilian coastal waters (Kraychete et al., 2022). Surprisingly, a fragment of IncU plasmid housing bla_{KPC-2} and *qnrVC1* was identified in a *K. quasipneumoniae* strain from surface water of this study. Although the *qnrVC* gene is commonly reported in *Vibrio* species (Pons et al., 2013), its presence on plasmids co-harboring β-lactamase-encoding genes in *Enterobacterales* seems to be advancing and deserves attention (Bado et al., 2018).

This study displayed how the high resolution provided by whole-genome sequencing allowed an in-depth understanding of the plasmid-mediated transmission of the bla_{KPC-2} gene to the environment. In this regard, whole-genome sequence-based analyzes should be implemented on a larger number of carbapenem-resistant strains in geographically distributed aquatic ecosystems. Consequently, it will be possible to monitor long-term national carbapenem resistance trends, as well as the evolution, prevalence, and transmission of antimicrobial resistance across different sectors.

5. Conclusions

Our findings highlight a diversity of plasmids harboring the bla_{KPC-2} gene embedded within Tn*4401* or NTE_{KPC} in XDR or MDR *K. pneumoniae* complex strains from aquatic ecosystems. Furthermore, our results evidence the long persistence of plasmids in Brazil, as well as the presence of uncommon plasmids, displaying the interspecies, intraspecies, and clonal transmission of carbapenem-resistant strains. Therefore, these data reinforce the importance of aquatic ecosystems in the dissemination and evolution of plasmid-mediated ARGs and contribute to One Health genomic surveillance studies of antimicrobial resistance.

Ethical approval

Not required.

CRediT authorship contribution statement

João Pedro Rueda Furlan: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Rafael da Silva Rosa:** Methodology, Formal analysis. **Micaela Santana Ramos:** Methodology, Formal analysis. **Lucas David Rodrigues dos Santos:** Methodology, Formal analysis. **Ralf Lopes:** Methodology, Formal analysis. **Eduardo Angelino Savazzi:** Conceptualization, Investigation. **Eliana Guedes Stehling:** Conceptualization, Investigation, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Data availability

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.163322.

References

Arcari, G., Carattoli, A., 2022. Global spread and evolutionary convergence of multidrug-resistant and hypervirulent Klebsiella pneumoniae high-risk clones. Pathog. Glob. Health 11, 1–14. https://doi.org/10.1080/20477724.2022.2121362.

Bado, I., Papa-Ezdra, R., Cordeiro, N., Outeda, M., Caiata, L., García-Fulgueiras, V., Seija, V., Vignoli, R., 2018. Detection of qnrVC6, within a new genetic context, in an NDM-1-producing citrobacter freundii clinical isolate from Uruguay. J. Glob. Antimicrob. Resist. 14, 95–98. https://doi.org/10.1016/j.jgar.2018.02.023.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19, 455–477. https://doi.org/10.1089/cmb.2012.0021.

Bonomo, R.A., Burd, E.M., Conly, J., Limbago, B.M., Poirel, L., Segre, J.A., Westblade, L.F., 2018. Carbapenemase-producing organisms: a global scourge. Clin. Infect. Dis. 66, 1290–1297. https://doi.org/10.1093/cid/cix893.

Brilhante, M., Gobeli Brawand, S., Endimiani, A., Rohrbach, H., Kittl, S., Willi, B., Schuller, S., Perreten, V., 2021. Two high-risk clones of carbapenemase-producing Klebsiella pneumoniae that cause infections in pets and are present in the environment of a veterinary referral hospital. J. Antimicrob. Chemother. 76, 1140–1149. https://doi.org/10.1093/jac/dkab028.

Brandt, C., Viehweger, A., Singh, A., Pletz, M.W., Wibberg, D., Kalinowski, J., Lerch, S., Müller, B., Makarewicz, O., 2019. Assessing genetic diversity and similarity of 435 KPC-carrying plasmids. Sci. Rep. 9, 11223. https://doi.org/10.1038/s41598-019-47758-5.

Cahill, N., O'Connor, L., Mahon, B., Varley, Á., McGrath, E., Ryan, P., Cormican, M., Brehony, C., Jolley, K.A., Maiden, M.C., Brisse, S., Morris, D., 2019. Hospital effluent: a reservoir for carbapenemase-producing Enterobacterales? Sci. Total Environ. 672, 618–624. https://doi.org/10.1016/j.scitotenv.2019.03.428.

Carattoli, A., 2013. Plasmids and the spread of resistance. Int. J. Med. Microbiol. 303, 298–304. https://doi.org/10.1016/j.ijmm.2013.02.001.

Chen, L., Mathema, B., Chavda, K.D., DeLeo, F.R., Bonomo, R.A., Kreiswirth, B.N., 2014. Carbapenemase-producing Klebsiella pneumoniae: molecular and genetic decoding. Trends Microbiol. 22, 686–696. https://doi.org/10.1016/j.tim.2014.09.003.

Chen, L., Chavda, K.D., Fraimow, H.S., Mediavilla, J.R., Melano, R.G., Jacobs, M.R., Bonomo, R.A., Kreiswirth, B.N., 2013. Complete nucleotide sequences of blaKPC-4- and blaKPC-5-harboring IncN and IncX plasmids from Klebsiella pneumoniae strains isolated in New Jersey. Antimicrob. Agents Chemother. 57, 269–276. https://doi.org/10.1128/AAC.01648-12.

Cherak, Z., Loucif, L., Moussi, A., Rolain, J.M., 2021. Carbapenemase-producing gram-negative bacteria in aquatic environments: a review. J. Glob. Antimicrob. Resist. 25, 287–309. https://doi.org/10.1016/j.jgar.2021.03.024.

Conlan, S., Thomas, P.J., Deming, C., Park, M., Lau, A.F., Dekker, J.P., Snitkin, E.S., Clark, T.A., Luong, K., Song, Y., Tsai, Y.C., Boitano, M., Dayal, J., Brooks, S.Y., Schmidt, B., Young, A.C., Thomas, J.W., Bouffard, G.G., Blakesley, R.W., NISC Comparative Sequencing Program, Mullikin, J.C., Korlach, J., Henderson, D.K., Frank, K.M., Palmore, T.N., Segre, J.A., 2014. Single-molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing Enterobacteriaceae. Sci Transl. Med. 6, 254ra126. https://doi.org/10.1126/scitranslmed.3009845.

Dallenne, C., Da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in enterobacteriaceae. J. Antimicrob. Chemother. 65, 490–495. https://doi.org/10.1093/jac/dkp498.

Cerdeira, L.T., Carneiro, A.R., Ramos, R.T., de Almeida, S.S., D'Afonseca, V., Schneider, M.P., Baumbach, J., Tauch, A., McCulloch, J.A., Azevedo, V.A., Silva, A., 2011. Rapid hybrid de novo assembly of a microbial genome using only short reads: corynebacterium pseudotuberculosis I19 as a case study. J. Microbiol. Methods 86, 218–223. https://doi.org/10.1016/j.mimet.2011.05.008.

De Gelder, L., Ponciano, J.M., Joyce, P., Top, E.M., 2007. Stability of a promiscuous plasmid in different hosts: no guarantee for a long-term relationship. Microbiology (Reading) 153 (Pt 2), 452–463. https://doi.org/10.1099/mic.0.2006/001784-0.

Feng, Y., Liu, L., McNally, A., Zong, Z., 2019. Coexistence of three blaKPC-2 genes on an IncF/IncR plasmid in ST11 Klebsiella pneumoniae. J. Glob. Antimicrob. Resist. 17, 90–93. https://doi.org/10.1016/j.jgar.2018.11.017.

Fonseca, E.L., Ramos, N.D., Andrade, B.G., Morais, L.L., Marin, M.F., Vicente, A.C., 2017. A one-step multiplex PCR to identify Klebsiella pneumoniae, klebsiella variicola, and klebsiella quasipneumoniae in the clinical routine. Diagn. Microbiol. Infect. Dis. 87, 315–317. https://doi.org/10.1016/j.diagmicrobio.2017.01.005.

Forster, B., Pinedo, C.A., 2015. Bacteriological examination of waters: membrane filtration protocol. American Society for Microbiology. https://asm.org/Protocols/Bacteriological-Examination-of-Waters-Membrane-Fi.

García-Betancur, J.C., Appel, T.M., Esparza, G., Gales, A.C., Levy-Hara, G., Cornistein, W., Vega, S., Nuñez, D., Cuellar, L., Bavestrello, L., Castañeda-Méndez, P.F., Villalobos-Vindas, J.M., Villegas, M.V., 2021. Update on the epidemiology of carbapenemases in Latin America and the Caribbean. Expert Rev. Anti-Infect. Ther. 19, 197–213. https://doi.org/10.1080/14787210.2020.1813023.

Ghiglione, B., Haim, M.S., Penzotti, P., Brunetti, F., D Amico González, G., Di Conza, J., Figueroa-Espinosa, R., Nuñez, L., Razzolini, M.T.P., Fuga, B., Esposito, F., Vander Horden, M., Lincopan, N., Gutkind, G., Power, P., Dropa, M., 2021. Characterization of emerging pathogens carrying blaKPC-2 gene in IncP-6 plasmids isolated from urban sewage in Argentina. Front. Cell. Infect. Microbiol. 11, 722536. https://doi.org/10.3389/fcimb.2021.722536.

Gillings, M.R., Westoby, M., Ghaly, T.M., 2018. Pollutants that replicate: xenogenetic DNAs. Trends Microbiol. 26, 975–977. https://doi.org/10.1016/j.tim.2018.08.003.

Hulme, P.E., 2020. One biosecurity: a unified concept to integrate human, animal, plant, and environmental health. Emerg. Top. Life Sci. 4, 539–549. https://doi.org/10.1042/ETLS20200067.

Hala, S., Antony, C.P., Alshehri, M., Althaqafi, A.O., Alsaedi, A., Mufti, A., Kaaki, M., Alhaj-Hussein, B.T., Zowawi, H.M., Al-Amri, A., Pain, A., 2019. First report of klebsiella quasipneumoniae harboring blaKPC-2 in Saudi Arabia. Antimicrob. Resist. Infect. Control 8, 203. https://doi.org/10.1186/s13756-019-0653-9.

Kraychete, G.B., Botelho, L.A.B., Monteiro-Dias, P.V., de Araújo, W.J., Oliveira, C.J.B., Carvalho-Assef, A.P.D., Albano, R.M., Picão, R.C., Bonelli, R.R., 2022. qnrVC occurs in different genetic contexts in klebsiella and enterobacter strains isolated from brazilian coastal waters. J. Glob. Antimicrob. Resist. 31, 38–44. https://doi.org/10.1016/j.jgar.2022.08.004.

Lopes, R., Furlan, J.P.R., Dos Santos, L.D.R., Stehling, E.G., 2022. Detection of CTX-M-27-positive endophytic Escherichia coli ST131 lineage C1/H30R subclade carrying blaKPC-2 on an IncX3-IncU plasmid in a fresh vegetable. J. Glob. Antimicrob. Resist. 30, 178–179. https://doi.org/10.1016/j.jgar.2022.06.012.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Munoz-Price, L.S., Poirel, L., Bonomo, R.A., Schwaber, M.J., Daikos, G.L., Cormican, M., Cornaglia, G., Garau, J., Gniadkowski, M., Hayden, M.K., Kumarasamy, K., Livermore, D.M., Maya, J.J., Nordmann, P., Patel, J.B., Paterson, D.L., Pitout, J., Villegas, M.V., Wang, H., Woodford, N., Quinn, J.P., 2013. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect. Dis. 13, 785–796. https://doi.org/10.1016/S1473-3099(13)70190-7.

Naas, T., Oueslati, S., Bonnin, R.A., Dabos, M.L., Zavala, A., Dortet, L., Retailleau, P., Iorga, B.I., 2017. Beta-lactamase database (BLDB) - structure and function. J. Enzyme Inhib. Med. Chem. 32, 917–919. https://doi.org/10.1080/14756366.2017.1344235.

Ni, W., Han, Y., Liu, J., Wei, C., Zhao, J., Cui, J., Wang, R., Liu, Y., 2016. Tigecycline treatment for carbapenem-resistant enterobacteriaceae infections: a systematic review and meta-analysis. Medicine (Baltimore) 95, e3126. https://doi.org/10.1097/MD.0000000000003126.

Larsson, D.G.J., Flach, C.F., 2022. Antibiotic resistance in the environment. Nat. Rev. Microbiol. 20, 257–269. https://doi.org/10.1038/s41579-021-00649-x.

Oliveira, S., Moura, R.A., Silva, K.C., Pavez, M., McCulloch, J.A., Dropa, M., Matté, M.H., Mamizuka, E.M., Sato, M.I., Pestana de Castro, A.F., Lincopan, N., 2014. Isolation of KPC-2-producing Klebsiella pneumoniae strains belonging to the high-risk multiresistant clonal complex 11 (ST437 and ST340) in urban rivers. J. Antimicrob. Chemother. 69, 849–852. https://doi.org/10.1093/jac/dkt431.

PAHO. Pan American Health Organization, 2021. Epidemiological alert: emergence and increase of new combinations of carbapenemases in Enterobacterales in Latin America and the Caribbean, 22 October 2021. PAHO/WHO, Washington, D.C. https://www.paho.org/en/documents/epidemiological-alert-emergence-and-increase-new-combinations-carbapenemases.

Peirano, G., Ahamed-Bentley, J., Woodford, N., Pitout, J.D., 2011. New Delhi metallo-β-lactamase from traveler returning to Canada. Emerg. Infect. Dis. 17, 240–242. https://doi.org/10.3201/eid1702.101313.

Pérez-Chaparro, P.J., Cerdeira, L.T., Queiroz, M.G., de Lima, C.P., Levy, C.E., Pavez, M., Lincopan, N., Gonçalves, E.C., Mamizuka, E.M., Sampaio, J.L., Nunes, M.R., McCulloch, J.A., 2014. Complete nucleotide sequences of two blaKPC-2-bearing IncN plasmids isolated from sequence type 442 Klebsiella pneumoniae clinical strains four years apart. Antimicrob. Agents Chemother. 58, 2958–2960. https://doi.org/10.1128/AAC.02341-13.

Piccirilli, A., Cherubini, S., Azzini, A.M., Tacconelli, E., Lo Cascio, G., Maccacaro, L., Bazaj, A., Naso, L., Amicosante, G., Group, Ltcf-Veneto Working, Perilli, M., 2021. Whole-Genome Sequencing (WGS) of carbapenem-resistant K. pneumoniae isolated in long-term care facilities in the Northern Italian Region. Microorganisms 9, 1985. https://doi.org/10.3390/microorganisms9091985.

Pitout, J.D., Gregson, D.B., Poirel, L., Mcclure, J.A., LE, P., Church, D.L., 2005. Detection of Pseudomonas aeruginosa producing Metallo-Beta-lactamases in a large centralized

laboratory. J. Clin. Microbiol. 43, 3129–3135. https://doi.org/10.1128/JCM.43.7.3129-3135.2005.

Polly, M., de Almeida, B.L., Lennon, R.P., Cortês, M.F., Costa, S.F., Guimarães, T., 2022. Impact of the COVID-19 pandemic on the incidence of multidrug-resistant bacterial infections in an acute care hospital in Brazil. Am. J. Infect. Control 50, 32–38. https://doi.org/10.1016/j.ajic.2021.09.018.

Pons, M.J., Gomes, C., Ruiz, J., 2013. QnrVC, a new transferable qnr-like family. Enferm. Infecc. Microbiol. Clin. 31, 191–192. https://doi.org/10.1016/j.eimc.2012.09.008.

Potter, R.F., D'Souza, A.W., Dantas, G., 2016. The rapid spread of carbapenem-resistant enterobacteriaceae. Drug Resist. Updat. 29, 30–46. https://doi.org/10.1016/j.drup.2016.09.002.

Rada, A.M., De La Cadena, E., Agudelo, C., Capataz, C., Orozco, N., Pallares, C., Dinh, A.Q., Panesso, D., Ríos, R., Diaz, L., Correa, A., Hanson, B.M., Villegas, M.V., Arias, C.A., Restrepo, E., 2020. Dynamics of blaKPC-2 dissemination from non-CG258 Klebsiella pneumoniae to other enterobacterales via IncN plasmids in an area of high endemicity. Antimicrob. Agents Chemother. 64. https://doi.org/10.1128/AAC.01743-20 e01743-.

Santos, W.M.D., Secoli, S.R., 2019. Economic burden of inpatients infected with Klebsiella pneumoniae carbapenemase. Einstein (Sao Paulo) 17, eGS4444. https://doi.org/10.31744/einstein_journal/2019GS4444.

Schmidt, J.S., Kuster, S.P., Nigg, A., Dazio, V., Brilhante, M., Rohrbach, H., Bernasconi, O.J., Büdel, T., Campos-Madueno, E.I., Gobeli Brawand, S., Schuller, S., Endimiani, A., Perreten, V., Willi, B., 2020. Poor infection prevention and control standards are associated with environmental contamination with carbapenemase-producing enterobacterales and other multidrug-resistant bacteria in swiss companion animal clinics. Antimicrob. Resist. Infect. Control 9, 93. https://doi.org/10.1186/s13756-020-00742-5.

Sellera, F.P., Fuga, B., Fontana, H., Esposito, F., Cardoso, B., Konno, S., Berl, C., Cappellanes, M.H., Cortez, M., Ikeda, M., de Souza, C.M., Cerdeira, L., Lincopan, N., 2021. Detection of IncN-pST15 one-health plasmid harbouring blaKPC-2 in a hypermucoviscous Klebsiella pneumoniae CG258 isolated from an infected dog, Brazil. Transbound. Emerg. Dis. 68, 3083–3088. https://doi.org/10.1111/tbed.14006.

Shropshire, W.C., Dinh, A.Q., Earley, M., Komarow, L., Panesso, D., Rydell, K., Gómez-Villegas, S.I., Miao, H., Hill, C., Chen, L., Patel, R., Fries, B.C., Abbo, L., Cober, E., Revolinski, S., Luterbach, C.L., Chambers, H., Fowler Jr., V.G., Bonomo Jr., R.A., Shelburne Jr., S.A., Kreiswirth Jr., B.N., van Duin Jr., D., Hanson Jr., B.M., Arias Jr., C.A., 2022. Accessory genomes drive independent spread of carbapenem-resistant Klebsiella pneumoniae clonal groups 258 and 307 in Houston, TX. MBio 13, e0049722. https://doi.org/10.1128/mbio.00497-22.

Souza, R.C., Dabul, A.N.G., Boralli, C.M.D.S., Zuvanov, L., Camargo, I.L.B.D.C., 2019. Dissemination of blaKPC-2 in an NTEKPC by an IncX5 plasmid. Plasmid 106, 102446. https://doi.org/10.1016/j.plasmid.2019.102446.

Stohr, J.J.J.M., Kluytmans-van den Bergh, M.F.Q., Weterings, V.A.T.C., Rossen, J.W.A., Kluytmans, J.A.J.W., 2021. Distinguishing blaKPC gene-containing IncF plasmids from epidemiologically related and unrelated enterobacteriaceae based on short- and long-read sequence data. Antimicrob. Agents Chemother. 65. https://doi.org/10.1128/AAC.00147-21 e00147-21.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E.M., Houchens, C.R., Grayson, M.L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., WHO Pathogens Priority List Working Group, 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect. Dis. 18, 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3.

Thomas, G.R., Corso, A., Pasterán, F., Shal, J., Sosa, A., Pillonetto, M., de Souza Peral, R.T., Hormazábal, J.C., Araya, P., Saavedra, S.Y., Ovalle, M.V., Jiménez Pearson, M.A., Chacón, G.C., Carbon, E., Mazariegos Herrera, C.J., Velásquez, S.D.C.G., Satan-Salazar, C., Villavicencio, F., Touchet, N.M., Busignani, S., Mayta-Barrios, M., Ramírez-Illescas, J., Vega, M.L., Mogdasy, C., Rosas, V., Salgado, N., Quiroz, R., El-Omeiri, N., Galas, M.F., Ramón-Pardo, P., Melano, R.G., 2022. Increased detection of carbapenemase-producing enterobacterales bacteria in Latin America and the Caribbean during the COVID-19 pandemic. Emerg. Infect. Dis. 28, 1–8. https://doi.org/10.3201/eid2811.220415.

UNEP, 2023. United Nations Environment Programme. Bracing for superbugs: strengthening environmental action in the One Health response to antimicrobial resistance. https://www.unep.org/resources/superbugs/environmental-action.

Van Duin, D., Kaye, K.S., Neuner, E.A., Bonomo, R.A., 2013. Carbapenem-resistant enterobacteriaceae: a review of treatment and outcomes. Diagn. Microbiol. Infect. Dis. 75, 115–120. https://doi.org/10.1016/j.diagmicrobio.2012.11.009.

Wang, X., Zhao, J., Ji, F., Chang, H., Qin, J., Zhang, C., Hu, G., Zhu, J., Yang, J., Jia, Z., Li, G., Qin, J., Wu, B., Wang, C., 2021. Multiple-replicon resistance plasmids of klebsiella mediate extensive dissemination of antimicrobial genes. Front. Microbiol. 12, 754931. https://doi.org/10.3389/fmicb.2021.754931.

Wyres, K.L., Holt, K.E., 2018. Klebsiella pneumoniae as a key trafficker of drug resistance genes from environmental to clinically important bacteria. Curr. Opin. Microbiol. 45, 131–139. https://doi.org/10.1016/j.mib.2018.04.004.

Yao, Y., Lazaro-Perona, F., Falgenhauer, L., Valverde, A., Imirzalioglu, C., Dominguez, L., Cantón, R., Mingorance, J., Chakraborty, T., 2017. Insights into a novel blaKPC-2-encoding IncP-6 plasmid reveal carbapenem-resistance circulation in several enterobacteriaceae species from wastewater and a hospital source in Spain. Front. Microbiol. 8, 1143. https://doi.org/10.3389/fmicb.2017.01143.

Zhu, Y., Liu, L., Schwarz, S., Liu, W., Wang, C., Yang, Q., Luan, T., Wang, L., Liu, S., Zhang, W., 2020. Characterization of a novel hybrid plasmid coharboring blaKPC-2 and qnrVC4 in a clinical citrobacter freundii strain. Antimicrob. Agents Chemother. 64. https://doi.org/10.1128/AAC.01379-20 e01379-20.