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Genome Note

Genomic data reveals the emergence of the co-occurrence of bla_{KPC-2} and $bla_{CTX-M-15}$ in an *Escherichia coli* ST648 strain isolated from rectal swab within the framework of hospital surveillance

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ABSTRACT

Objectives: The worldwide dissemination of carbapenemase-producing *Escherichia coli* lineages belonging to high-risk clones poses a challenging public health menace. The aim of this work was to investigate genomic features of a colonizing multidrug-resistant strain of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *E. coli* from our institution.

Methods: Whole-genome sequencing was done by Illumina MiSeq-I, and *de novo* assembly was achieved using SPAdes. Resistome, mobilome, plasmids, virulome, and integrons were analysed using ResFinder, AMRFinder, ISFinder, PlasmidFinder, MOB-suite, VirulenceFinder, and IntegronFinder. Sequence types (STs) were identified with pubMLST and BIGSdb databases. Conjugation assays were also performed.

Results: *Escherichia coli* HA25pEc was isolated from a rectal swab sample taken within the framework of the hospital epidemiological surveillance protocol for detection of carbapenemase-producing *Enterobacterales*. *Escherichia coli* HA25pEc corresponded to the first report of ST648 co-harbouring bla_{KPC-2} and $bla_{CTX-M-15}$ in Latin America from a colonized patient. It had 19 antibiotic resistance genes (ARGs), including bla_{KPC-2}, located on a Tn*4401a* isoform. Conjugation assays revealed that bla_{KPC-2} was not transferred by conjugation to *E. coli* J53 under our experimental conditions.

Conclusion: *Escherichia coli* ST648 has been detected previously in companion and farm animals as well as in hospital- and community-acquired infections worldwide. Although scarcely reported as KPC-producers, our finding in a culture surveillance with several acquired ARGs, including $bla_{CTX-M-15}$, alerts the potential of this clone for worldwide unnoticed spreading of extreme drug resistance to β-lactams. These data reinforce the importance of carrying out molecular surveillance to identify reservoirs and warn about the dissemination of new international clones in carbapenemase-bearing patients.

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Table 1
Antimicrobial resistance profile and genetic determinants found in *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Escherichia coli* ST648 HA25pEc strain, *E. coli* ECO44, *E. coli* ECO67, and *E. coli* Ec351 strains.

Strain name	*E. coli* HA25pEc	*E. coli* ECO44	*E. coli* ECO67	*E. coli* Ec351
Type	Colonizing strain	Infecting strain	Infecting strain	Infecting strain
Isolation country	Argentina	Argentina	Argentina	Brazil
Sequence type	ST648	ST648	ST648	ST648
Draft genomes				
Contigs	257	376	354	246
Genome sequence	5,329,253	5,285,682	5,233,480	5,318,322
N50 contig size	164,144	114,441	118,298	101,142
GC% Average	50.7	50.41	50.56	50.3
Number of CDS	5121	5111	5081	5049
Number of RNAs	103	49	56	48
Antimicrobial Resistance Profile	S: AN, CL, CZA, FM, FOS, GM, TGC R: CAZ, CIP, CRO, ETP, FEP, IMI, MEM, MIN, SXT, PTZ	S: AN, CL, FOS, GM, TGC R: ATM, CAZ, CIP, CTX, ETP, FEP, MEM, PTZ, SXT	S: AN, CIP, CL, FOS, GM, TGC R: ATM, CAZ, CTX, ETP, FEP, IMI, MEM, PTZ, SXT	S: CHL, GM, FOS R: AN, ATM, CAZ, CIP, CRO, CTX, CZ, ETP, FEP, IMI, MEM, SXT
ARG with 100% QC and ID to already described	*aac(6′)-Ib* (M21682); *aadA5* (AF137361); *aph(4)-Ia* (V01499); *aph(3′)-III* (V01499); *bla*$_{KPC-2}$ (AY034847); *bla*$_{CTX-M-15}$ (AY044436); *dfrA14* (KF921535); *dfrA17* (FJ460238); *sul1* (U12338); *sul2* (AY034138); *mph(A)* (D16251)	*aac(6′)-Ib-cr* (DQ303918); *aadA5* (AF137361); *aph(3′′)-Ib* (AF321551); *aph(6′)-Id* (CP000971); *bla*$_{CMY-2}$ (X91840); *bla*$_{KPC-2}$ (AY034847); *bla*$_{OXA-1}$ (HQ170510); *bla*$_{TEM-1B}$ (AY458016); *catB3* (U13880); *dfrA17* (FJ460238); *mph(A)* (D16251); *qacEΔ1* (X68232); *sul1* (U12338); *sul2* (AY034138); *tet(A)* (AJ517790)	*aph(3′′)-Ib* (AF321551); *aph(6′)-Id* (CP000971); *bla*$_{KPC-2}$ (AY034847); *bla*$_{TEM-1B}$ (AY458016); *dfrA17* (FJ460238); *sul1* (U12338); *tet(B)* (AF326777)	*aadA5* (AF137361); *bla*$_{CTX-M-15}$ (AY044436); *bla*$_{KPC-2}$ (AY034847); *dfrA17* (FJ460238); *qnrB19* (EU432277); *sul1* (U12338); *tet(B)* (AF326777)
ARG with > 100% QC and/or ID	*aac(3)-IV* (DQ241380); *aph(3′)-Ib* (AF321551); *bla*$_{OXA-9}$ (KQ089875); *bla*$_{TEM-1A}$ (HM749966); *mdf(A)* (Y08743); *bla*$_{OXA-9}$ (WP_000722315); *bla*$_{TEM-1}$ (WP_000027057)			*erm(B)* (JN899585); *aph(3′)-VIa* (X07753); *mph(A)* (D16251)
Mobile elements	IS*100* (AJ851089); IS*1203* (U06468); IS*1F* (X52538); IS*1H* (U15127); IS*1R* (J01730); IS*1S* (M37615); IS*30* (X00792); IS*Aeme7* (QVI02670); IS*682* (NC_002695); IS*903* (J01839); IS*911* (X17613); IS*Ec1* (L02370); IS*Ec31* (NC_011751); IS*Ec38* (AJ303141); IS*Ec59* (KX246266); IS*Ecp1* (AJ242809); IS*Kpn13* (EU780013); IS*Kpn14* (CP000649); IS*Kpn6* (UXL13710); IS*Kpn7* (AMQ45718); MITE*Ec1* (U00096); Tn*3* (KT378596); Tn*4401* (KT378596)	IS*100* (Z32853); IS*4* (J01733); IS*5* (J01735); IS*26* (X00011); IS*Apu1* (CR376602); IS*Ec17* (DQ388534); IS*Ec30* (NC_011603); IS*Ec81* (NC_010498); IS*Ecp1* (AJ242809); IS*Kox3* (CP003684); IS*Kpn6* (UXL13710); IS*Kpn8* (EF382672); IS*Kpn27* (NC_016846); MITE*Ec1* (U00096)	IS*30* (X00792); IS*4* (J01733); IS*5075* (AF457211); IS*Apu1* (CR376602); IS*Ec30* (NC_011603); IS*Ec38* (AJ303141); IS*Ec81* (NC_010498); IS*Kox3* (CP003684); IS*Kpn6* (UXL13710); IS*Kpn8* (EF382672); IS*Kpn27* (NC_016846); MITE*Ec1* (U00096); Tn*6196* (KC999035)	IS26 (X00011); IS*4* (J01733); IS*6100* (X53635); IS*Ec9* (AJ242809); IS*Ec30* (NC_011603); IS*Ec38* (AJ303141); IS*Ec81* (NC_010498); IS*Kpn6* (UXL13710); IS*Kpn8* (EF382672); IS*Kpn27* (NC_016846); MITE*Ec1* (U00096)

(*continued on next page*)

Table 1 (*continued*)

Strain name	*E. coli* HA25pEc	*E. coli* ECO44	*E. coli* ECO67	*E. coli* Ec351
Main virulence genes	*air* (CP003034); *chuA* (UARX01000012); *eilA* (CP000970); *fyuA* (ADTP01000030); *hlyE* (ECU57430); *irp2* (NZ_NMCD01000015); *iucC* (CXZO01000056); *iutA* (CXZO01000056); *kpsE* (AAMK02000004); *kps* (MG739441); *lpfA* (CP003034); *neuC* (JJLW01000144); *ompT* (CP015834); *papA* (AF247354); *papC* (DQ010312); *sitA* (ADTK01000286); *terC* (CP007491); *traT* (CXZC01000023); *yfcV* (UFZN01000001)	*chuA* (UARX01000012); *fimH* (JX847135); *fyuA* (ADTP01000030); *irp2* (NZ_NMCD01000015); *kpsMII* (CP015834)	*chuA* (UARX01000012); *fimH* (JX847135); *fyuA* (ADTP01000030); *irp2* (NZ_NMCD01000015); *iucC* (CXZO01000056); *iutA* (CXZO01000056); *kpsMII* (CP015834)	*agn43* (WP_000820498); *air* (CP003034); *aslA* (WP_000395844); *cea* (QONK01000239); *chuATUVSWY*; *ecpABCDER;* *eil*A (CP003034); *elf*A (WP_000742542); *entBCDEFS;* *espLRXY;* *fedC* (WP_063085018); *fepABCDG;* *fes* (NP_415117); *fimABCDEFGHI;* *flu* (WP_000820498); *focX* (CAM84401); *fyuA* (ADTP01000030); *gad* (CP001671); *gspCDEFGHIJKLM;* *irp1* (WP_000369488); *irp2* (NZ_NMCD01000015); *iss* (CP001846); *kpsDEM;* *lpfA* (CP003034); *matA* (WP_000389022); *ompA* (WP_022645481); *ompT* (CP015834); *papX* (KAF0950165); *shuAX;* *stgA* (AAS99229); *traT* (CXZO01000046); *tssABCDFGLJHM;* *ybtAEPQSTUX;* *ycbF* (WP_160483575)
Plasmid replicons	Col (MG828), ID: 95, QC: 99.23; IncFIA (AP001918), ID:99.74, QC: 100; IncFIB (AP001918), ID: 98.39, QC: 100; IncFII (pHN7A8), ID: 96.54, QC: 100; IncFII (pRSB107), ID: 100, QC: 100; IncR (DQ449578), ID: 100, QC: 100	IncFII (AY458016), ID: 100, QC: 100; IncL/M(pMU407) (U27345), ID: 99.59, QC: 100	IncFII (pRSB107) (AJ851089), ID: 100, QC: 100; IncL/M (pMU407) (U27345), ID: 99.59, QC: 100; IncFIB(AP001918), ID: 98.39, QC: 100; Incl1 (AP005147), ID: 97.89, QC: 100; IncFIA (AP001918), ID: 99.74, QC: 100	IncX4 (CP002895), ID: 100, QC: 100; IncFIA (AP001918), ID: 99.74, QC: 100; IncFIB (AP001918), ID: 98.39, QC: 100; IncFII (CP024805.1), ID: 98.08, QC: 100; IncQ1 (M28829), ID: 81.28, QC: 100
Plasmid carrying bla_{KPC-2}	pDCMP1-KPC (IncR)	IncL/M	IncL/M	pEc351 (IncQ1)
Conjugation assays	Non-conjugative	Not known	Not known	Not known

NOTE: A total of 16 antimicrobial agents were tested in *E. coli* HA25pEc by BD Phoenix automated system. Susceptibility to colistin in *E. coli* HA25pEc was done by the pre-diffusion method according to the National Antimicrobial Reference Laboratory, Malbrán Institute, INEI-ANLIS, Argentina (http://antimicrobianos.com.ar/ATB/wp-content/uploads/2017/09/Protocolo-Predifusion-Tabletas-COL-Rosco-version2-Agosto2017.pdf). MOB-suite (https://github.com/phac-nml/mob-suite) was used to identify replicon types in *E. coli* HA25pEc. Each plasmid FASTA file generated by MOB-suite was analysed using PLSDB (https://academic.oup.com/nar/article/47/D1/D195/5149885, https://ccb-microbe.cs.uni-saarland.de/plsdb/), PlasmidFinder (https://cge.food.dtu.dk/services/PlasmidFinder/), AMRFinder (https://www.ncbi.nlm.nih.gov/pathogens/antimicrobial-resistance/AMRFinder/), and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). PLSDB predicted replicons in *E. coli* HA25pEc with an identity to plasmid reference greater than 99% were used to confirm MOB-suite results. Notice that the plasmid sequences from *E. coli* HA25pEc are not circular and were predicted from the draft assemblies by MOB-suite. Data from *E. coli* ECO44 and *E. coli* ECO67 were obtained from Sanz et al. [2], and data from *E. coli* Ec351 were obtained from Fuga et al. [3]. Genomic information not available in the mentioned publications was obtained through bioinformatical analysis in the present work.

AN, amikacin; ATM, aztreonam; CAZ, ceftazidime; CDS, coding sequence; CHL, chloramphenicol; CIP, ciprofloxacin; CL, colistin; CRO, ceftriaxone; CTX, cefotaxime; CZ, cefazolin; CZA, ceftazidime-avibactam; ETP, ertapenem; FEP, cefepime; FM, nitrofurantoin; FOS, fosfomycin; GM, gentamicin; ID, identity; IMI, imipenem; MEM, meropenem; PTZ, piperacillin-tazobactam; QC, query cover; R, resistant; S, sensitive; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline.

Escherichia coli is naturally harboured in the intestinal microbiota of humans and other animals [1]. Although they are usually not dangerous, part of their population can become extraintestinal pathogenic *E. coli* (ExPEC) strains [1,2]. ExPEC strains can cause a great number of human diseases, both in health care-associated and community settings [2]. A limited number of lineages of ExPEC are responsible for these infections; the pandemic multidrug-resistant *E. coli* clones described so far belong to ST10, ST38, ST69, ST131, ST155, ST224, ST393, ST405, ST410, and ST648 [2,3]. The population structure of *E. coli* is predominantly clonal, and strains belong to one of eight phylogenetic groups: A, B1, B2, C, D, E, F, and G [1]. Accordingly, each phylogroup is associated to the lifestyle of the strains, including proclivity to cause a particular disease.

Recently, genomic study on 71 carbapenemase-producing ExPEC strains isolated from July 2008 and March 2017 from nosocomial settings from Argentina revealed large clonal diversity, with clonal complex (CC) CC10 isolates from phylogroup A being the most abundant followed by CC131 from phylogroup B2, in which some also co-produce CTX-M-15 [2]. Contrasting the A and B2 phylogroups, the clinical significance of *E. coli* strains from phylogroup F has not yet been investigated in-depth [4,5]. Phylogroup F includes ST648, which can cause infections in animals and humans. *In silico*, *in vitro*, and *in vivo* analysis demonstrated that it is well-equipped with biofilm-associated features that may contribute to its successful emergence worldwide across different ecologies [5]. It has been suggested that it should be recognized as a high-risk food-borne pathogen [4].

The epidemiological success of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *E. coli* has been related to pandemic lineages capable of disseminating plasmids that carry the bla_{KPC-2} gene in the genetic platform of the mobile element Tn*4401* or related structures [3]. ExPEC strains disseminate the bla_{KPC-2} gene most frequently in plasmids of the IncF type, followed by IncN, IncX, IncA/C, IncP, IncL/M, IncR, IncH, IncI, IncU, and Col [3]. Recently, an IncQ1 small plasmid carrying bla_{KPC-2} in *E. coli* ST648 was identified in Brazil [3].

Escherichia coli HA25pEc was isolated from a rectal swab sample taken on the first day of hospitalization from patient 25, within the framework of a hospital epidemiological surveillance protocol. It was obtained from a 72-year-old man with a history of long-term home hospitalization for terminal chronic obstructive pulmonary disease who was admitted to our hospital due to traumatic hemarthrosis. The minimal inhibitory concentrations (MICs) of the tested antimicrobials were determined with a BD Phoenix Automated Microbiology System and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022) (Table 1). Because *E. coli* HA25pEc was a producer of KPC, contact precaution was installed to prevent horizontal transmission. One week later, the patient developed an infection of the urinary tract and a *K. pneumoniae* strain susceptible to all antibiotics tested was isolated. He completed 7 days of ampicillin-sulbactam with clinical improvement. Total genomic sequencing was performed by Illumina's MiSeq-I technology. Whole-genome sequencing, *de novo* assembly, bioinformatics analysis, and conjugation assays (Table 1) were performed as described previously [6]. Multilocus sequence typing classified HA25pEc as *E. coli* ST648. ResFinder, AMRFinder, and PlasmidFinder revealed that *E. coli* HA25pEc ST648 possessed several replicons (Table 1) and corresponded to the first report of *E. coli* ST648 co-harbouring both bla_{KPC-2} and $bla_{CTX-M-15}$ genes in Argentina and the first isolate as a colonizing strain from Latin America. *Escherichia coli* HA25pEc had 19 antibiotic resistance genes (ARGs) (Table 1). MOB-suite identified that the contig NODE_7, which contained $bla_{CTX-M-15}$, corresponded to a fragment located in the chromosome. BLASTN (Nucleotide Basic Local Alignment Search Tool) searches using the sequence of NODE_7 as query were used to confirm chromosome location of $bla_{CTX-M-15}$. As result, three *E. coli* chromosomes (CP034389.1, CP109874.1, and CP033850.1) were retrieved with 100% query cover and 100% identity (data not shown) that confirmed the chromosomal location. VirulenceFinder (https://cge.food.dtu.dk/services/VirulenceFinder/) predicted 19 genes related to virulence in *E. coli* (Table 1). The bla_{KPC-2} gene was found in an IncR replicon; the putative plasmid, pDCMP1-KPC, was not transferred by conjugation assays to *E. coli* J53. The bla_{KPC-2} gene was identified in Tn*4401a* with 100% coverage and 99.99% identity with the structure previously found in *K. pneumoniae* HA3 isolated from our institution (Supplementary Fig. S1, Table 1, JAMQEI010000039.1). Recently, two clinical *E. coli* ST648 strains, *E. coli* ECO44 and ECO67 from Argentina, have been described as harbouring the bla_{KPC-2} gene in a Tn*3* variant Ia/b genetic platform [2]; also, one *E. coli* Ec351 ST648 strain from Brazil was found on an NTEKPC-IId element carrying bla_{KPC-2} [3] (Supplementary Fig. S1). As a main difference with previously described *E. coli* ST648 strains from our country, this strain harboured $bla_{CTX-M-15}$ (Table 1). A genomic description of these three KPC-producing *E. coli* ST648 strains compared with *E. coli* HA25pEc ST648 is summarized in Table 1. Analysis of *E. coli* HA25pEc with ISFinder (https://isfinder.biotoul.fr/) revealed 21 insertion sequences (ISs), 2 transposons, and 2 miniature inverted repeat transposable elements (MITEs) (Table 1).

Because the dissemination of the bla_{KPC-2} gene among strains of *E. coli* belonging to ST648 has been described as mediated mostly by large plasmids IncN, IncC, and IncHI2, as well as a small plasmid, IncQ1 [3], the finding of bla_{KPC-2} in an IncR plasmid in *E. coli* ST648 HA25pEc represents another difference with previous reports from Latin America. KPC-producing *E. coli* ST648 strains have been identified in China, Greece, the United States, Colombia, Brazil, and recently, in Argentina [2,3] (Table 1). The *E. coli* HA25pEc strain was isolated in the frame of the epidemiological surveillance culture that was carried out by protocol of the infection control program from our institution, which was not etiologically responsible for the patient's infection. *Escherichia coli* ST648 has been detected in animals (companion and farm) and in infections of hospitalized and community patients globally. Our results reinforce the importance of carrying out molecular surveillance to identify reservoirs and alert about the dissemination of new international clones that may go unnoticed in carbapenemase-bearing patients.

Nucleotide sequence accession numbers

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAOTOG000000000. The version described in this paper is version JAOTOG010000000.

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Competing interests

None declared.

Ethical approval

Not required.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.12.012.

References

[1] Johnston BD, Thuras P, Porter SB, Anacker M, VonBank B, Vagnone PS, Witwer M, Castanheira M, Johnson JR, et al. Global molecular epidemiology of carbapenem-resistant Escherichia coli (2002-2017). Eur J Clin Microbiol Infect Dis 2021 Epub ahead of print. PMID: 34278542. doi:10.1007/s10096-021-04310-6.

[2] Sanz MB, De Belder D, de Mendieta JM, Faccone D, Poklepovich T, Lucero C, et al. Carbapenemase-producing extraintestinal pathogenic *Escherichia coli* from Argentina: clonal diversity and predominance of hyperepidemic clones CC10 and CC131. Front Microbiol 2022;13:1–11. doi:10.3389/fmicb.2022.830209.

[3] Fuga B, Cerdeira L, Moura Q, Fontana H, Fuentes-Castillo D, Carvalho AC, et al. Genomic data reveals the emergence of an IncQ1 small plasmid carrying bla_{KPC-2} in *Escherichia coli* of the pandemic sequence type 648. J Glob Antimicrob Resist 2021;25:8–13. doi:10.1016/j.jgar.2021.02.014.

[4] Zhuge X, Zhou Z, Jiang M, Wang Z, Sun Y, Tang F, et al. Chicken-source *Escherichia coli* within phylogroup F shares virulence genotypes and is closely related to extraintestinal pathogenic *E. coli* causing human infections. Transbound Emerg Dis 2021;68:880–95. doi:10.1111/tbed.13755.

[5] Schaufler K, Semmler T, Wieler LH, Trott DJ, Pitout J, Peirano G, et al. Genomic and functional analysis of emerging virulent and multidrug-resistant *Escherichia coli* lineage sequence type 648. Antimicrob Agents Chemother 2019;63. doi:10.1128/AAC.00243-19.

[6] Álvarez VE, Massó MG, D'amico González G, Gambino AS, Knecht CA, Prack Mc Cormick B, Leguina C, Piekar M, Poklepovich T, Campos J, Arduino S, Centrón D, Quiroga MP. Emergence of colistin resistance in *Klebsiella pneumoniae* ST15 disseminating bla_{KPC-2} in a novel genetic platform. J Glob Antimicrob Resist 2022;29:537–9 Epub 2021 Dec 9. PMID: 34896335. doi:10.1016/j.jgar.2021.12.001.