Journal of Global Antimicrobial Resistance 32 (2023) 85–87

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Genome Note

Replacement of KPC-producing pandemic lineages and dissemination of plasmids associated with antimicrobial resistance determinants during inpatient's hospitalization

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ARTICLE INFO

Article history:
Received 15 June 2022
Revised 12 October 2022
Accepted 21 October 2022
Available online 8 November 2022

Editor: Stefania Stefani

Keywords:
Klebsiella pneumoniae ST258
Klebsiella pneumoniae ST11
Carbapenem resistance
bla_{KPC-2}

ABSTRACT

Objectives: The emergence of bla_{KPC-2} within nosocomial settings has become a major public health crisis worldwide. Our aim was to perform whole-genome sequencing (WGS) of three KPC-producing Gram-negative bacilli (KPC-GNB) strains isolated from a hospitalized patient to identify acquired antimicrobial resistance genes (ARGs).
Methods: WGS was performed using Illumina MiSeq-I, and *de novo* assembly was achieved using SPAdes. Bioinformatics analysis was done using Resfinder, AMRFinder, ISFinder, plasmidSPAdes, PlasmidFinder, MOB-suite, PLSDB database, and IntegronFinder. Conjugation assays were performed to assess the ability of bla_{KPC-2} to transfer via a plasmid-related mobilization mechanism.
Results: High-risk clone KPC-producing *Klebsiella pneumoniae* sequence type (ST) 258 (HA3) was colonizing an inpatient who later was infected by KPC-producing *Escherichia coli* ST730 (HA4) and subsequently by KPC-producing *K. pneumoniae* ST11 (HA15) during hospitalization. Although belonging to different species, both strains causing infections harbored the same gene configuration for dissemination of bla_{KPC-2} in related IncM1 plasmids recently found in other KPC-GNB isolated from Hospital Alemán at Ciudad Autónoma de Buenos Aires. Conjugation assays revealed that only pDCVEA4-KPC from *E. coli* HA4 was successfully transferred with a conjugation frequency of 3.66×10^{1}.
Conclusions: Interchange of multidrug-resistant *K. pneumoniae* lineages ST258 replaced by ST11 in the framework of colonization and infection by KPC-GNB of an inpatient from our institution was found. In addition, the transfer of the gene configuration of bla_{KPC-2} between infecting strains may have occurred in the nosocomial environment, but we cannot rule out that the event took place *in vivo*, within the patient, during hospitalization.

Although studies focusing on antimicrobial treatments and early detection of *Klebsiella pneumoniae* carbapenemase (KPC) could have helped mitigate the effect of KPC-producing *Enterobacterales*, this remains as a global threat to public health [1–3]. Argentina has been endemic for KPC since 2010, mainly due to widespread of KPC-producing *K. pneumoniae* (KPC-Kp) ST258 and other clonal types, including ST11 [1,2]. Also, KPC-producing *Escherichia coli* (KPC-Ec) ST10 and ST131 have been recently identified in clinical isolates from Argentina [2]. Our goal was to perform whole-genome sequencing (WGS) of three KPC-producing strains isolated in 2019 from an inpatient at Hospital Alemán from Ciudad Autónoma de Buenos Aires to identify acquired antimicrobial resistance genes (ARGs). The inpatient was female and 66 years of age with stage IV ovarian cancer, treated with chemotherapy and surgery for intestinal obstruction, who was readmitted with an episode of febrile neutropenia. On the first day of hospitalization as part of a surveillance program, a carbapenemase-producing

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https://doi.org/10.1016/j.jgar.2022.10.016

Table 1
Antimicrobial resistance profiles and genetic determinants found in KPC-producing *Klebsiella pneumoniae* and *Escherichia coli* strains. A total of 18 antimicrobial agents were tested by BD Phoenix Automated Microbiology System (BD Biosciences, Sparks, MD, USA).

STRAIN NAME	*K. pneumoniae* HA3	*E. coli* HA4	*K. pneumoniae* HA15
TYPE	colonizing	infecting	infecting
SEQUENCE TYPE	ST258	ST730	ST11
DRAFT GENOMES			
CONTIGS	151	105	152
GENOME SEQUENCE	5 670 773 bp	4 880 265 bp	5 583 198 bp
N50 CONTIG SIZE	176 763 bp	216 883 bp	157 077 bp
GC% AVERAGE	57.1%	50.8%	57.2%
NUMBER OF CDS	5,490	4,686	5,344
NUMBER OF RNAS	119	124	118
ANTIMICROBIAL RESISTANCE PROFILE	**S:** TGC, CZA, CL **R:** AM, AMC, CZ, CRO, CAZ, FEP, PTZ, IMI, MEM, ETP, GM, AK, CIP, SXT, FOS, FM	**S:** GM, AN, CIP, SXT, FOS, CL, FM **R:** AM, AMC, CZ, CRO, CAZ, FEP, PTZ, IMI, MEM, ETP	**S:** GM, AN, SXT, FOS, CL **R:** AM, AMC, CZ, CRO, CAZ, FEP, PTZ, IMI, MEM, ETP, CIP, FM
ARGs WITH 100% QC AND ID	*aac*(3)-IV (**DQ241380**), *aac*(6′)-Ib (**M21682**), *aadA1* (**JQ414041**), *aph(3′)-Ia* (**V00359**), *aph(4)-Ia* (**WP_000742814.1**), bla_{KPC-2} (**AY034847**), *dfrA12* (**AM040708**), *mph(A)* (**D16251**), *sul1* (**U12338**), *sul3* (**AJ459418**), *catA1* (**WP_000412211.1**), *qacL* (**WP_000800531.1**), *cmlA1* (**WP_000095725.1**)	bla_{KPC-2} (**AY034847**)	*oqxA* (**WP_002914189.1**), bla_{KPC-2} (**WP_004199234.1**), *aph(3′)-Ia* (**WP_000018329.1**)
ARGs WITH LESS THAN 100% QC AND/OR ID	*fosA* (**WP_004146118.1**), bla_{OXA-9} (**WP_000722315.1**), bla_{TEM-1} (**WP_000027057.1**), *aadA2* (**WP_001206356.1**)	*mdf(A)* (**Y08743**)	*fosA* (**WP_004146118.1**), *oqxB* (**WP_000347934.1**)
MAIN VIRULENCE GENES	*fyuA* (**EGF64092**), *irp1* (**HBW1015239**), *irp2* (**WP_155034160**), *iutA* (**CDO13951**), *mrkA* (**WP_065810031.1**), *mrkB* (**HCI6327958**), *mrkC* (**HCI6179678**), *mrkD* (**WP_095285098**), *mrkF* (**USP91422**), *mrkH* (**WP_004152886**), *mrkI* (**SYE32402**), *mrkJ* (**WP_128317782**), *ybtA* (**HBR9486812**), *ybtE* (**MCN4084224**), *ybtP* (**HBX5790681**), *ybtQ* (**WP_001446633**), *ybtS* (**WP_000703040**), *ybtT* (**CAD1952594**), *ybtU* (**WP_104443368**), *ybtX* (**WP_213075853**)	*ompT* (**KJ26851**), *terC* (**ALY14307**), *hcpA* (**WP_000360895),** *hcpB* (**WP_001402015**), *hcpC* (**WP_000157236**), *fimA* (**WP_000695543**), *fimB* (**WP_250297566**), *fimC* (**WP_001438970**), *fimD* (**WP_032283229**), *fimE* (**WP_191997666**), *fimF* (**EEC26037**), *fimG* (**WP_001162240**), *fimH* (**ALY16044**), *fimI* (**WP_077252771**), *ibeB* (**WP_000074254**), *ibeC* (**WP_000556304**), *cfaA* (**WP_000225867**)	*fyuA* (**EGF64092**), *irp1* (**HBW1015239**), *irp2* (**WP_155034160**), *iutA* (**CDO13951**), *mrkA* (**WP_065810031.1**), *mrkB* (**HCI6327958**), *mrkC* (**HCI6179678**), *mrkD* (**WP_095285098**), *mrkF* (**USP91422**), *mrkH* (**WP_004152886**), *mrkI* (**SYE32402**), *mrkJ* (**WP_128317782**), *ybtA* (**HBR9486812**), *ybtE* (**MCN4084224**), *ybtP* (**HBX5790681**), *ybtQ* (**WP_001446633**), *ybtS* (**WP_000703040**), *ybtT* (**CAD1952594**), *ybtU* (**WP_104443368**), *ybtX* (**WP_213075853**)
PLASMID REPLICONS	ColRNAI (**DQ298019,** ID:100%, QC:100%) IncFIB(K) (**JN233704,** ID:100%, QC:100%) IncFII(K) (**CP000648,** ID:100%, QC:100%) IncR (**DQ449578,** ID:100%, QC:100%) IncX3 (**JN247852,** ID:100%, QC:100%)	IncI2(Delta)(**AP002527,** ID:100%, QC:100%) IncM1(**U27345,** ID:99.59%, QC:100%)	Col440I (**CP023920,** ID: 97.37%, QC: 100%) Col440I (**CP023920,** ID: 96.49%, QC: 100%) IncFIB(K) (**JN233704,** ID: 100%, QC: 100%) IncFII(K) (**CP000648,** ID: 100%, QC: 100%) IncM1(**MN626603,** ID: 99.98%, QC: 83%)
PLASMIDS CARRYING bla_{KPC-2}	pDCVEA3-KPC (IncR)	pDCVEA4-KPC (IncM1)	pDCVEA15-KPC (IncM1)
CONJUGATION ASSAYS	Non-conjugative	Conjugative	Non-conjugative

NOTE: Each plasmid FASTA file generated by MOB-suite was analyzed using PLSDB (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 with an identity to plasmid reference greater than 99% were used to confirm MOB-suite results. Virulence genes were found using the BIGSdb database (https://bigsdb.pasteur.fr/klebsiella/) and VirulenceFinder (https://cge.cbs.dtu.dk/services/VirulenceFinder/). MOB-suite (https://github.com/phac-nml/mob-suite) was used to identify replicon types.

AM, ampicillin; AMC, amoxicillin clavulanic acid; AN, amikacin; CAZ, ceftazidime; CDS, coding sequences; CRO, ceftriaxone; CZ, cefazolin; CIP, ciprofloxacin; CL, colistin; CZA, ceftazidime-avibactam; ETP, ertapenem; FEP, cefepime; FOS, fosfomycin; GM, gentamicin; ID, identity; IMI, imipenem; MEM, meropenem; FM, nitrofurantoin; PTZ, piperacillin-tazobactam; QC, query cover; R, resistant; S, sensitive; SXT, trimethoprim-sulfadiazine; TGC, tigecycline.

K. pneumoniae colonizing strain, HA3, was isolated from a rectal swab. Twenty-three and 70 days after hospitalization, an *E. coli* HA4 strain and a *K. pneumoniae* HA15 strain, respectively, were isolated from urine samples taken for suspected urinary tract infection. All strains showed resistance to imipenem, meropenem, ertapenem, ceftriaxone, ceftazidime, and cefepime (Table 1). The minimal inhibitory concentrations (MICs) of the tested antimicrobials were determined with a BD Phoenix Automated Microbiology System and interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines (Supplementary Table S1).

WGS, *de novo* assembly, bioinformatics analysis, and conjugation assays (Table 1) were performed as previously described [3]. Multilocus sequence typing classified KPC-Kp HA3, KPC-Ec HA4, and KPC-Kp HA15 as ST258, ST730, and ST11, respectively. A total of 17, 2, and 5 transferable ARGs (Table 1) were identified in KPC-Kp HA3, KPC-Ec HA4, and KPC-Kp HA15, respectively. Interestingly, analysis of the flanking sequences of the bla_{KPC-2} gene revealed that infecting KPC-Ec HA4 and KPC-Kp HA15 shared the same gene configuration as Tn*3*-*tnpA*-IS*Apu1*-IS*Apu2*-IS*Kpn27*-bla_{KPC-2}-IS*Kpn6*-Tn3-Tn*10* (18176 bp), previously found in KPC-Kp ST18 (HA7pKpn) and *Enterobacter hormachei* ST45 (HA2pEhor) strains isolated from the same hospital (JAMPTY000000000.1 and JAMQJX000000000.1, respectively). Instead, the bla_{KPC-2} gene harbored by the colonizing KPC-Kp HA3 strain was inserted in a complete Tn*4401a* transposon as Tn*5403tnpA*-Tn*552bin3*-IS*5tnpA*-IS*Kpn31tnpA*-IS*Kpn6*-bla_{KPC-2}-IS*Kpn7*-IS*Psy42tnpA*-*xerD.* This structure showed 100% identity and query cover with the genetic configuration found in *E. coli* Ecol_244 strain from Argentina (CP019017.1), as well as with those of *K. pneumoniae* BWHC1 (CP020500.1), *K. pneumoniae* KPNIH24 (CP008798.1), *K. pneumoniae* 38544 (CP010362.1), and *K. pneumoniae* 34618 (CP010396.1) strains from the United States. The KPC-Kp HA3 strain possessed bla_{KPC-2} in an IncR plasmid (pDCVEA3-KPC), while in infecting KPC-Ec HA4 and KPC-Kp HA15 strains, bla_{KPC-2} was in an IncM1 plasmid (pDCVEA4-KPC and pDCVEA15-KPC, respectively) (Table 1). Further bioinformatics analysis of pDCVEA4-KPC using the Basic Local Alignment Search Tool (BLAST) revealed that it was identical to pDCCK1-KPC, recently described in *E. hormaechei* HA2pEhor (NZ_JAMQJX010000016.1) and *K. pneumoniae* HA7pKpn strains (NZ_JAMPTY010000024.1). Moreoever, pDCVEA15-KPC had 100% identity and 99% query cover with pDCCK1-KPC. Transfer of bla_{KPC-2} from KPC-Kp HA3, KPC-Ec HA4, and KPC-Kp HA15 to *E. coli* J53 was carried out by biparental conjugation. Only pDCVEA4-KPC from *E. coli* HA4 was transferred with a conjugation frequency of 3.66×10^{1}. Transconjugant strains showed resistance to imipenem, meropenem, ertapenem, and ceftazidime, and the presence of bla_{KPC-2} was confirmed by polymerase chain reaction (PCR).

In this work, three findings, likely related to the success of the worldwide spreading of bla_{KPC-2}, have been found. First, exchange of pandemic multidrug-resistant KPC-Kp lineages (ST258 replaced by ST11) was found in the framework of colonization and infection of one inpatient from our institution. KPC-Kp ST11 is common in Asia, but recent studies have found that it is disseminating in Argentina [1]. Because hypervirulent clones of *K. pneumonaie* ST11 have been reported [4], our results emphasize that KPC-Kp ST11 could have biological advantages over KPC-Kp ST258 that led to its success during infection processes. Secondly, KPC-Ec HA4 represents not only the first isolate of KPC-Ec ST730 harbouring bla_{KPC-2} in a conjugative IncM1 plasmid alerting its ability to disseminate, but also the first report of this lineage disseminating in Latin America, evidencing that spread through novel lineages may represent an additional global burden in the context of increasing antimicrobial resistance. Previously, *E. coli* ST730 was found in China harbouring $bla_{CTX-M-14}$ and bla_{OXA-30} (CP027202.2), and in Switzerland related to bovine mastitis [5]. Lastly, transfer of the gene configuration harboring bla_{KPC-2}, as well as microevolution of plasmids pDCVEA4-KPC and pDCVEA15-KPC, was documented between our infecting strains. It is likely that these rearrangements occurred in the nosocomial environment, but it cannot be ruled out that the events took place *in vivo*, within the patient, during hospitalization. Our results showed that prospective studies to investigate KPC-producing strains can contribute to the understanding of molecular features related to worldwide propagation and on the prevention of further dissemination.

Nucleotide sequence accession numbers

The Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the accessions **JAMQEI000000000, JAMQEJ000000000**, and **JAMQEK000000000**. The versions described in this paper are **JAMQEI010000000, JAMQEJ010000000**, and **JAMQEK010000000**. Plasmids pDCVEA3-KPC, pDCVEA4-KPC, and pDCVEA15-KPC were registered at GenBank with the accession numbers **NZ_JAMQEI010000039.1, NZ_JAMQEJ010000022.1**, and **NZ_JAMQEK010000029.1**, respectively. Notice that the plasmid sequences are not circular and were predicted from the draft assemblies by MOB-suite.

Funding

The authors' work was supported by grants ISID/Pfizer 2019 given to DC, PUE 2522 from CONICET given to IMPaM (UBA/CONICET), and UBACYT Programación Científica 2018 given to DC and MPQ. DC and MPQ are members of the Carrera del Investigador Científico, CONICET, Argentina. VEA has a Postdoctoral Fellowship from CONICET. ASG is a recipient of a CONICET doctoral fellowship. MP is a recipient of an Universidad de Buenos Aires's doctoral fellowship. NGA is an infectious diseases doctor. BF and LFC are clinical microbiology specialists.

Competing interests

None declared

Ethical approval

Not required

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.10.016.

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