Journal of Global Antimicrobial Resistance 30 (2022) 178–179

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

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

Detection of CTX-M-27-positive endophytic *Escherichia coli* ST131 lineage C1/*H*30R subclade carrying bla_{KPC-2} on an IncX3-IncU plasmid in a fresh vegetable

Editor: Stefania Stefani

Sir,

Broad-spectrum cephalosporins- and carbapenem-resistant Enterobacterales are a leading cause of nosocomial infections. In this regard, *Escherichia coli* ST131 is a high-risk extraintestinal pathogenic lineage with three major clades (A, B, and C) [1]. Moreover, a rapidly emerging epidemic sublineage carrying $bla_{CTX-M-27}$ is recognised as a distinct cluster, named C1-M27, within the C1/*H*30R subclade [2,3], but has not been reported from vegetables so far. Here, we report the detection of a KPC-2-producing endophytic *E. coli* ST131-C1-M27 strain colonising internal tissues of a fresh vegetable and raising a food safety alert about KPC-2 producers.

During a surveillance study conducted to detect antimicrobial-resistant Enterobacterales in crops, fresh vegetables were collected from a farm with a history of cow manure use in Brazil. For surface sterilization, leaves were washed sequentially in 70% ethanol, sodium hypochlorite (2.5% chlorine), 30% hydrogen peroxide, 37% formaldehyde, and five times in sterile distilled water. To confirm the sterilization protocol, aliquots of the sterile water used in the final rinse were plated onto nutrient agar. For the isolation of endophytic bacteria, surface-sterilized leaves were cut into 1 cm^2 fragments and then mixed thoroughly in saline solution. Subsequently, the suspension was plated onto MacConkey agar supplemented with meropenem (2 mg/L) or ceftriaxone (2 mg/L).

A carbapenem-resistant endophytic *E. coli* strain, named P11, was isolated from a chicory sample. Antimicrobial susceptibility testing was carried out using disk diffusion followed by MIC determination by agar dilution method for all antimicrobials tested, except for colistin, determined by broth microdilution method (CLSI 2021, supplement M100, 31st ed.). In this regard, P11 strain was resistant to critically important antimicrobials (i.e., aminoglycosides, fluoroquinolones, third- and fourth-generation cephalosporins, and carbapenems), while susceptibility to colistin was retained (Table 1).

For whole-genome sequencing, total DNA was extracted using the GenElute^{TM} Bacterial Genomic DNA Kit (Sigma-Aldrich, USA) according to the manufacturer's instructions. A genomic library was constructed using a Nextera XT DNA Library Preparation Kit (Illumina, UK), and whole-genome sequencing was performed using an Illumina HiSeq platform (Illumina, USA), generating 308 × coverage of P11 genome. *De novo* assembly was performed using SPAdes v. 3.15.2. Multilocus sequence typing (MLST), serotype, FimH-type, resistome, virulome, and plasmid replicons were analysed using bioinformatics tools available at the Center for Genomic Epidemiology (http://www.genomicepidemiology.org/). In addition, plasmid contigs were assembled using plasmidSPAdes v. 3.15.2, and the preliminary scaffold was refined with Geneious v. 11.1.5. BLASTn analyses were performed to verify the similarity of P11 contigs to plasmid sequences at GenBank. Gap sizes were estimated, and, using a combined strategy, we conducted gap closure by multiple plasmid alignment and iterative anchoring of short reads by craning to gap flanks. Additionally, replication and termination regions were identified from the DoriC 5.0 database (http://tubic.tju.edu.cn/doric/public/index.php/index).

P11 strain belonged to serotype O25b:H4 and ST131/clonal complex 131, a globally disseminated clone at the human-animal-environment interface and mainly associated with CTX-M variants [2,4]. Regarding virulence, our strain belonged to phylotype B2, and the following genes were detected: *gad* (glutamate decarboxylase), *iha* (adherence protein), *iss* (increased serum survival), *iucC* (aerobactin synthetase), *ompT* (outer membrane protease), *sat* (secreted autotransporter toxin), *senB* (enterotoxin), and *yfcV* (fimbrial protein), similar to clinical *E. coli* strains of ST131 and C1-M27 subclade [2]. Antimicrobial resistance genes (ARGs) to β-lactams ($bla_{CTX-M-27}$, bla_{KPC-2}), aminoglycosides [*aadA1, aadA2, aadA2b, aadA5, aph(3'')-Ib, aph(6)-Id*], sulphonamides (*sul1, sul2, sul3*), trimethoprim (*dfrA12, dfrA17*), tetracyclines (*tetA*), macrolides (*mdfA, mphA*), phenicols (*cmlA1*), and quaternary ammonium compounds (*qacE*Δ*1, qacH2*), as well as amino acid substitutions in the quinolone resistance-determining region of GyrA (Ser83Leu, Asp87Asn), ParC (Ser80Ile, Glu84Val), and ParE (Ile529Leu), were also identified.

E. coli P11 harboured two plasmids, ~135 kb (named pP11a) and ~75 kb (named pP11b), bearing $bla_{CTX-M-27}$ and bla_{KPC-2}, respectively, which were transferred separately by conjugation using sodium azide–resistant *E. coli* J53 as recipient strain. Transconjugants were selected using MacConkey agar supplemented with sodium azide (200 mg/L) and ceftriaxone (2 mg/L), or sodium azide (200 mg/L) and meropenem (2 mg/L), and confirmed by PCR for the detection of ARGs (Table 1). The conjugation frequencies of pP11a and pP11b were 5.76×10^{-3} and 1.45×10^{-2} transconjugants/recipient, respectively.

The pP11a plasmid belonged to IncF [F1:A2:B20] and was 135 152 bp in length, containing 53.4% GC and 167 coding regions (CDSs). In addition to $bla_{CTX-M-27}$, this plasmid co-harboured *aadA5, aph(3'')-Ib, aph(6)-Id, sul1, sul2, dfrA17, tetA, mphA*, and *qacE*Δ*1* genes and showed a high similarity (76%–79% query coverage and ~98.5% nucleotide identity) to other F1:A2:B20 plasmids of *E. coli* ST131-C1-M27 from different countries (GenBank accession nos. CP051616.1, LC520270.1, LR890300.1, and MG886288.1).

Otherwise, the pP11b plasmid was a 75 816 bp IncX3-IncU plasmid with 52.7% GC and 109 CDSs. Besides bla_{KPC-2}, pP11b co-harboured the In*649* integron carrying the *dfrA12-gcuF-aadA2-aadA2b-cmlA1-aadA1-qacH2* gene cassette array associated with *sul3* (GenBank accession no. EF113389.1). This plasmid was most related (44% query coverage and 99.2% nucleotide identity) to

https://doi.org/10.1016/j.jgar.2022.06.012
2213-7165/© 2022 The Authors. Published by Elsevier Ltd on behalf of International Society for Antimicrobial Chemotherapy. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Table 1
Genetic and microbiological features of *E. coli* P11 and its transconjugants

	P11	J53-pP11a	J53-pP11b	J53
Resistance genes	*bla*$_{CTX-M-27}$, *bla*$_{KPC-2}$, *aadA1, aadA2, aadA2b, aadA5, aph(3'')-Ib, aph(6)-Id, sul1, sul2, sul3, dfrA12, dfrA17, tetA, mdfA, mphA, cmlA1, qacE*Δ*1, qacH2*	*bla*$_{CTX-M-27}$, *aadA5, aph(3'')-Ib, aph(6)-Id, sul1, sul2, dfrA17, tetA. mphA, qacE*Δ*1*	*bla*$_{KPC-2}$, *aadA1, aadA2, aadA2b, sul3, dfrA12, cmlA1, qacH2*	–
Plasmid replicon types	IncF [F1:A2:B20], IncX3-IncU	IncF [F1:A2:B20]	IncX3-IncU	–
MIC (mg/L)[a]				
Ampicillin	≥256[b]	≥256[b]	≥256[b]	1
Ampicillin/sulbactam	128/64[b]	32/16[b]	64/32[b]	2/4
Ceftazidime	64[b]	16[b]	32[b]	0.5
Ceftriaxone	≥256[b]	64[b]	≥256[b]	≤0.25
Cefotaxime	≥256	64	≥256[b]	≤0.25
Cefepime	32[b]	16[b]	16[b]	≤0.25
Aztreonam	64[b]	16[b]	32[b]	0.125
Imipenem	64[b]	0.5	64[b]	0.5
Meropenem	32[b]	≤0.06	32[b]	≤0.06
Gentamicin	128[b]	64[b]	32[b]	0.5
Ciprofloxacin	128[b]	≤0.03	≤0.03	≤0.03
Tetracycline	≥256[b]	≥256[b]	1	1
Chloramphenicol	≥256[b]	2	64[b]	1
Colistin	1	0.5	0.5	0.5

[a] Resistance profiles were determined using the CLSI 2021 guideline (supplement M100, 31st ed.).
[b] MIC value indicating resistance.

IncX3-IncU plasmids harbouring *bla*$_{KPC-2}$ of *Klebsiella pneumoniae* and *E. coli* isolated from human patients in Brazil and the United States (GenBank accession nos. CP003997.1, CP027700.1, KU963389.1, MF150120.1, and MK264770.1).

Analysis of *bla*$_{CTX-M-27}$ genetic context revealed the presence of insertion sequences flanking the ARG gene by ΔIS*Ecp1* (upstream, 208 bp length) and ΔIS*903D* (downstream, 385 bp length), both disrupted by IS*26*. In this regard, IS*Ecp1* and IS*26* mobilised progenitors of *bla*$_{CTX-M}$ genes, including *bla*$_{CTX-M-27}$, onto plasmids [5–7]. Additionally, the *bla*$_{KPC-2}$ gene was harboured by a non-Tn*4401* element (NTE$_{KPC}$) classified as NTE$_{KPC}$-Ic, presenting a partial IS*Kpn6* with the associated left inverted repeat and a Tn*3* resolvase gene (*tnpR*) downstream and upstream, respectively, of *bla*$_{KPC-2}$.

In conclusion, to the best of our knowledge, we report the first detection of KPC-2-producing *E. coli* ST131-C1-M27 from a fresh vegetable. Although the origin of *bla*$_{CTX-M-27}$ and *bla*$_{KPC-2}$ in crops was unclear, cattle manure and irrigation water might be the most likely sources. Therefore, continued monitoring of critical priority bacteria in crops remains necessary for the early identification of high-risk clones, thus contributing to the prevention of outbreaks.

Nucleotide sequence accession numbers

Nucleotide sequences of *E. coli* P11 have been deposited at GenBank under accession numbers: JAHUZJ000000000.1 (whole-genome shotgun sequencing project), MZ513634.1 (plasmid pP11a), and MZ513635.1 (plasmid pP11b).

Funding

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (grant numbers 88887.464733/2019-00, 88882.180855/2018-01, and Finance code 001) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (grant numbers 2018/01890-3 and 2021/01655-7).

Competing interests

None declared.

Ethical approval

Not required.

References

[1] Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, et al. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci USA 2014;111:5694–9. doi:10.1073/pnas.1322678111.
[2] Matsumura Y, Pitout JD, Gomi R, Matsuda T, Noguchi T, Yamamoto M, et al. Global *Escherichia coli* sequence type 131 clade with *bla*$_{CTX-M-27}$ gene. Emerg Infect Dis 2016;22:1900–7. doi:10.3201/eid2211.160519.
[3] Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 2017;72:2145–55. doi:10.1093/jac/dkx146.
[4] Lopes R, Furlan JPR, Dos Santos LDR, Gallo IFL, Stehling EG. Colistin-resistant *mcr-1*-positive *Escherichia coli* ST131-*H22* carrying *bla*$_{CTX-M-15}$ and *qnrB19* in agricultural soil. Front Microbiol 2021;12:659900. doi:10.3389/fmicb.2021.659900.
[5] Humeniuk C, Arlet G, Gautier V, Grimont P, Labia R, Philippon A. β-lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-encoded CTX-M types. Antimicrob Agents Chemother 2002;46:3045–9. doi:10.1128/AAC.46.9.3045-3049.2002.
[6] Amos GC, Hawkey PM, Gaze WH, Wellington EM. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J Antimicrob Chemother 2014;69:1785–91. doi:10.1093/jac/dku079.
[7] Matsuo N, Nonogaki R, Hayashi M, Wachino JI, Suzuki M, Arakawa Y, et al. Characterization of *bla*$_{CTX-M-27}$/F1:A2:B20 plasmids harbored by *Escherichia coli* sequence type 131 sublineage C1/H30R isolates spreading among elderly Japanese in nonacute-care settings. Antimicrob Agents Chemother 2020;64:e00202–20. doi:10.1128/AAC.00202-20.

Ralf Lopes
João Pedro Rueda Furlan
Lucas David Rodrigues dos Santos
Eliana Guedes Stehling*
Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil
*Corresponding author.
E-mail address: elianags@usp.br (E.G. Stehling)
Revised 20 May 2022