



Draft Genome Sequence of a *bla*_{KPC-2}-Carrying *Citrobacter braakii* Isolate from Pediatric Hospital Wastewater in Peru

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ABSTRACT Here, we report a draft genome sequence of a *bla*_{KPC-2}-carrying *Citrobacter braakii* isolate from pediatric hospital effluent. Genome CF248 represents a multidrug-resistant *C. braakii* isolate derived from a clinical environment in Peru.

Citrobacter is a motile, facultative, anaerobic member of the *Enterobacteriaceae* associated with nosocomial infections (1, 2). The emergence of *Citrobacter* members as nosocomial pathogens causing urinary and respiratory tract infections has been described (3–6), along with reports of clinical isolates carrying horizontally transferred antibiotic resistance genes (ARGs), such as *bla*_{KPC-2} (7). Hospital water effluents may act as reservoirs for ARG-carrying bacteria, including carbapenemase-producing *Enterobacterales* (8). Routine monitoring of wastewater can detect resistant pathogens released into municipal wastewater and natural water bodies (8, 9).

We recovered isolate CF248 in December 2017 as part of a pilot program to monitor hospital effluent from a pediatric hospital in Lima, Peru. The precipitate of a 50-ml water sample collected from an exit sewage pipe was swabbed onto MacConkey agar (Becton, Dickinson, Heidelberg, Germany) and incubated for 24 h at 37°C. One lactose-fermenting colony was restreaked onto MacConkey agar and classified as a *Citrobacter* sp. with the Phoenix automated microbiology system (BD Diagnostic Systems, Sparks, MD, USA). We identified resistance to 11 different antibiotics, including ertapenem, imipenem, and meropenem (Table 1), following CLSI guidelines (10). KPC production was confirmed with the RESIST-4 OKNV (Coris BioConcept, Gembloux, Belgium) immunochromatographic rapid diagnostic test for carbapenemase detection.

Genomic DNA was extracted from 1 ml of tryptic soy broth (Becton, Dickinson) single-colony culture incubated at 37°C for 6 hours, using the GeneJET genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA, USA). Nextera XT libraries (Illumina, Inc., San Diego, CA, USA) were sequenced using a MiSeq instrument generating 1,735,120 paired-end 250-bp reads. Reads were processed and analyzed for ARG detection with the Nullarbor v.2.0 pipeline (11), using SPAdes (v.3.15.2) (12) and Prokka (v.1.13.3) (13) for assembly and annotation, respectively, resulting in a draft genome of 294 contigs larger than 300 bp (N_{50} , 49,265 bp) and a total length of 5,810,531 bp, with a 64× mean coverage and a GC content of 52.3%. Plasmid replicon typing was performed using plasmidfinder v.2.0 (14), which is available at the Center of Genomic Epidemiology (www.genomicepidemiology.org). Antibiotic resistance genes and plasmid replicons with a cutoff value of >95% for the identity and a coverage of 100% were included. Default parameters were used for all software tools.

Resistome analysis identified ARGs for aminoglycosides [*aph*(3')-VIa], fluoroquinolones (*qnrB68*), and β-lactams (*bla*_{CMY-83} and *bla*_{KPC-2}). We identified plasmids of incompatibility (Inc) groups H11A (CIT), H11B (CIT), FIB (K), and P6. The plasmid replicon IncP6 has been

Citation Salvatierra G, Dávila-Barclay A, Ayzanoa B, Cuicapuza D, Santillán-Salas C, Tsukayama P. 2021. Draft genome sequence of a *bla*_{KPC-2}-carrying *Citrobacter braakii* isolate from pediatric hospital wastewater in Peru. *Microbiol Resour Annot* 10:e00569-21. <https://doi.org/10.1128/MRA.00569-21>.

Editor David A. Baltus, University of Arizona

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Received 2 June 2021

Accepted 9 July 2021

Published 29 July 2021

TABLE 1 Antimicrobial susceptibility testing of the *Citrobacter braakii* CF248 isolate

Antibiotic	MIC ($\mu\text{g/ml}$)	Interpretation ^a
Aminoglycosides		
Amikacin	≤ 8	S
Gentamicin	≤ 2	S
Penicillins		
Ampicillin	> 16	R
Penicillins + β -lactamase inhibitor		
Ampicillin-sulbactam	$> 16/8$	R
Piperacillin-tazobactam	$> 64/4$	R
Cephalosporins		
Cefazolin	> 8	R
Cefepime	8	I
Cefoxitin	> 16	R
Ceftazidime	16	I
Ceftriaxone	> 4	R
Fluoroquinolones		
Ciprofloxacin	> 2	R
Levofloxacin	> 4	R
Sulfonamides		
Trimethoprim-sulfamethoxazole	$\leq 0.5/9.5$	S
Glycylcyclines		
Tigecycline	2	S
Carbapenems		
Ertapenem	> 1	R
Imipenem	8	R
Meropenem	8	R

^a S, susceptible; I, intermediate; R, resistant.

reported as a carrier of *bla*_{KPC-2} in *Pseudomonas aeruginosa* (15), *Klebsiella oxytoca*, *Enterobacter cloacae*, *Citrobacter freundii* (16), and *Citrobacter braakii* (17). Contig assessment revealed that the *bla*_{KPC-2} gene was located on a Tn3 transposon element of 13.8 kb with the linear structure ISKpn27- Δ *bla*_{TEM-1}-*bla*_{KPC-2}-ISKpn6-*korC*-*kICA*-*repB*. BLAST analysis of the *bla*_{KPC-2} gene sequence showed 100% sequence identity and 100% query coverage with the 111,195-bp plasmid pKPHS2 (accession number NC_016846) isolated from a clinical *bla*_{KPC-2}-carrying *Klebsiella pneumoniae* isolate in China.

Here, we report a *bla*_{KPC-2}-producing *C. braakii* isolate derived from hospital wastewater in Peru. Our results suggest that antibiotic resistance monitoring of hospital wastewater may detect antimicrobial resistance (AMR) dissemination from clinical settings into the sewage environment.

Data availability. The draft genome assembly of *Citrobacter braakii* CF248 has been submitted to NCBI under accession number GCA_018449455.1 under BioProject PRJNA729854, and the raw reads have been submitted to the SRA under accession number SRR14534310.

ACKNOWLEDGMENTS

This study was funded by the Innóvate Perú grant number 289-2017 and FONDECYT grant number 088-2018. G.S. was supported by the CONCYTEC-FONDECYT-World Bank Group grant number E033-01-08-2018-FONDECYT/Banco Mundial-Programas de Doctorado en Áreas Estratégicas y Generales.

REFERENCES

- Liu LH, Wang NY, Wu AYJ, Lin CC, Lee CM, Liu CP. 2018. *Citrobacter freundii* bacteremia: risk factors of mortality and prevalence of resistance genes. *J Microbiol Immunol Infect* 51:565–572. <https://doi.org/10.1016/j.jmii.2016.08.016>.
- Zhang R, Yang L, Jia CC, Hong WZ, Chen GX. 2008. High-level carbapenem resistance in a *Citrobacter freundii* clinical isolate is due to a combination of KPC-2 production and decreased porin expression. *J Med Microbiol* 57:332–337. <https://doi.org/10.1099/jmm.0.47576-0>.

3. Mohanty S, Singhal R, Sood S, Dhawan B, Kapil A, Das BK. 2007. Citrobacter infections in a tertiary care hospital in Northern India. *J Infect* 54:58–64. <https://doi.org/10.1016/j.jinf.2006.01.015>.
4. Jiménez A, Castro JG, Muñoz-Price LS, de Pascale D, Shimose L, Mustapha MM, Spychala CN, Mettus RT, Cooper VS, Doi Y. 2017. Outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Citrobacter freundii* at a tertiary acute care facility in Miami, Florida. *Infect Control Hosp Epidemiol* 38:320–326. <https://doi.org/10.1017/ice.2016.273>.
5. Hammerum AM, Hansen F, Nielsen HL, Jakobsen L, Stegger M, Andersen PS, Jensen P, Nielsen TK, Hansen LH, Hasman H, Fuglsang-Damgaard D. 2016. Use of WGS data for investigation of a long-term NDM-1-producing *Citrobacter freundii* outbreak and secondary in vivo spread of blaNDM-1 to *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*. *J Antimicrob Chemother* 71:3117–3124. <https://doi.org/10.1093/jac/dkw289>.
6. Pletz MW, Wollny A, Dobermann UH, Rödel J, Neubauer S, Stein C, Brandt C, Hartung A, Mellmann A, Trommer S, Edel B, Patchev V, Makarewicz O, Maschmann J. 2018. A nosocomial foodborne outbreak of a VIM carbapenemase-expressing *Citrobacter freundii*. *Clin Infect Dis* 67:58–64. <https://doi.org/10.1093/cid/ciy034>.
7. Wu W, Espedido B, Feng Y, Zong Z. 2016. *Citrobacter freundii* carrying blaKPC-2 and blaNDM-1: characterization by whole genome sequencing. *Sci Rep* 6:30670. <https://doi.org/10.1038/srep30670>.
8. Cahill N, O'Connor L, Mahon B, Varley Á, McGrath E, Ryan P, Cormican M, Brehony C, Jolley KA, Maiden MC, 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>.
9. Urase T, Okazaki M, Tsutsui H. 2020. Prevalence of ESBL-producing *Escherichia coli* and carbapenem-resistant Enterobacteriaceae in treated wastewater: a comparison with nosocomial infection surveillance. *J Water Health* 18:899–910. <https://doi.org/10.2166/wh.2020.014>.
10. Clinical and Laboratory Standards Institute. 2020. Performance standard for antimicrobial susceptibility testing. M100, 30th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Seemann T, Goncalves da Silva A, Bulach DM, Schultz MB, Kwong JC, Howden BP. 2019. Nullarbor Github. <https://github.com/tseemann/nullarbor>.
12. Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. 2020. Using SPAdes de novo assembler. *Curr Protoc Bioinformatics* 70:e102. <https://doi.org/10.1002/cpbi.102>.
13. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
14. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. In silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
15. Dai X, Zhou D, Xiong W, Feng J, Luo W, Luo G, Wang H, Sun F, Zhou X. 2016. The IncP-6 plasmid p10265-KPC from *Pseudomonas aeruginosa* carries a novel ΔISEc33-associated blaKPC-2 gene cluster. *Front Microbiol* 7:310. <https://doi.org/10.3389/fmicb.2016.00310>.
16. 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>.
17. Dong D, Mi Z, Li D, Gao M, Jia N, Li M, Tong Y, Zhang X, Zhu Y. 2020. Novel IncR/IncP6 hybrid plasmid pCRE3-KPC recovered from a clinical KPC-2-producing *Citrobacter braakii* isolate. *mSphere* 5:e00891-19. <https://doi.org/10.1128/mSphere.00891-19>.