



Draft Genome Sequences of *Citrobacter freundii* and *Citrobacter murliniae* Strains Isolated from the Feces of Preterm Infants

Yuhao Chen,^a Thomas C. Brook,^b Cristina Alcon-Giner,^c Paul Clarke,^d  Lindsay J. Hall,^c  Lesley Hoyles^e^aDepartment of Surgery and Cancer, Imperial College London, London, United Kingdom^bDepartment of Biomedical Sciences, University of Westminster, London, United Kingdom^cGut Microbes and Health Programme, Quadram Institute Bioscience, Norwich, United Kingdom^dNeonatal Intensive Care Unit, Norfolk and Norwich University Hospital, Norwich, United Kingdom^eDepartment of Biosciences, Nottingham Trent University, Nottingham, United Kingdom

ABSTRACT Here, we describe the draft genome sequences of three strains of *Citrobacter* isolated from feces of preterm neonates with suspected sepsis. Strains P106E PI and P079F I were *Citrobacter freundii*. Strain P080C CL represents the first draft genome sequence of *Citrobacter murliniae*.

Species of the genus *Citrobacter* are considered members of the human gut microbiota and are opportunistic pathogens in a range of nosocomial infections (1). Worldwide, they are associated with neonatal sepsis in a subset of infants, and multidrug-resistant strains are being detected with increasing frequency (2–6).

Fecal samples were collected from three preterm neonates with suspected sepsis. Briefly, after storage at -80°C , fecal samples were diluted 1:10 in TBT buffer (100 mM Tris/HCl [pH 8.0], 100 mM NaCl, and 10 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) and plated onto MacConkey agar no. 3 and incubated overnight at 37°C to isolate lactose-positive (pink) colonies (7). Details for the sources of the strains described here can be found in Table 1. Phenotypic testing (API 20E) identified the strains as *Citrobacter* sp. DNA was extracted using a phenol-chloroform method described fully by Kiu et al. (8) from overnight cultures of strains and sequenced using the 96-plex Illumina HiSeq 2500 platform to generate 125-bp paired-end reads (9). Raw data provided by the sequencing center were checked using FastQC v0.11.4 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>); no adapter trimming was required, and reads had an average Phred score of >25 . MetaPhlan2.6 (10) was used to identify the closest relatives of strains, leading to a reference-based (*Citrobacter freundii* complex strain MGH104; Assembly accession no. [GCA_001034485](https://doi.org/10.1128/MRA.00494-19)) assembly being produced by BugBuilder v1.0.3b1 (default settings for Illumina assembly) (11). Summary statistics for the genome sequences are given in Table 1, with completeness (99.9, 99.9, and 100%, respectively) determined using CheckM v1.0.13 (12). Genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (13). BLASTP analysis of the proteomes of the three strains against Comprehensive Antibiotic Resistance Database (CARD) data v3.0.1 (<https://card.mcmaster.ca/latest/data>) (14) using the recommended bit score cutoffs for strict matches (gene dependent) showed the strains to encode a range of antibiotic resistance determinant homologs, with two strains encoding β -lactamases and one encoding PmrF, which is linked to colistin resistance (Fig. 1A).

FastANI (15) was used to determine the average nucleotide identity (ANI) of the genomes against that of the type strain, NCTC 9750^T, of *C. freundii* (Assembly accession no. [GCA_900635425](https://doi.org/10.1128/MRA.00494-19)). P106E PI and P079F I were confirmed to be *Citrobacter freundii* (98.6% and 98.7% ANI, respectively) (16–18). Multilocus sequence typing showed P079F

Citation Chen Y, Brook TC, Alcon-Giner C, Clarke P, Hall LJ, Hoyles L. 2019. Draft genome sequences of *Citrobacter freundii* and *Citrobacter murliniae* strains isolated from the feces of preterm infants. *Microbiol Resour Announc* 8:e00494-19. <https://doi.org/10.1128/MRA.00494-19>.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

Copyright © 2019 Chen et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Lindsay J. Hall, Lindsay.Hall@quadram.ac.uk, or Lesley Hoyles, lesley.hoyles@ntu.ac.uk.

Received 14 May 2019

Accepted 22 July 2019

Published 15 August 2019

TABLE 1 Clinical information and genome sequence statistics for the three *Citrobacter* strains

Strain	Source of feces	Genome information							
		No. of reads	Size (bp)	No. of contigs	Coverage (×)	N_{50} (bp)	No. of CDS ^a	No. of tRNAs	G+C content (%)
P079F I	12-day-old male; Caesarean section (gestational age, 30 wks); wt, 1,544 g	989,778	5,273,335	64	47	261,533	5,056	71	51.8
P080C CL	12-day-old male; vaginal delivery (gestational age, 25 wks, 5 days); wt, 831 g	1,132,580	5,024,923	59	56	260,081	4,647	69	50.6
P106E PI	10-day-old female; vaginal delivery (gestational age, 30 wks, 4 days); wt, 1,402 g	1,149,416	5,139,193	106	56	178,284	4,840	72	51.3

^aCDS, coding sequences.

I to be sequence type 311 (ST311) and P106E PI to be ST95. Strain P080C CL was assigned as a *Citrobacter* sp. by MetaPhlan2.6, so its 16S rRNA gene sequence was identified within the whole-genome sequence using RNAmmer v1.2 (19) and compared against 16S rRNA gene sequences available at EzBioCloud (<https://www.ezbiocloud.net/>) (20). It shared 100% similarity with *Citrobacter murlinae* CDC2970-59^T. To determine whether P080C CL represented a strain of *C. murlinae*, sequence reads deposited for the type strain, CIP 104556 (1), were downloaded from the Sequence Read Archive (accession no. [ERR664250](https://www.ncbi.nlm.nih.gov/seq/ERR664250)) and assembled using SPAdes v3.11.1 (default settings) (21) for inclusion in ANI analyses (Fig. 1B). Strain P080C CL shared 99.3% ANI with *C.*

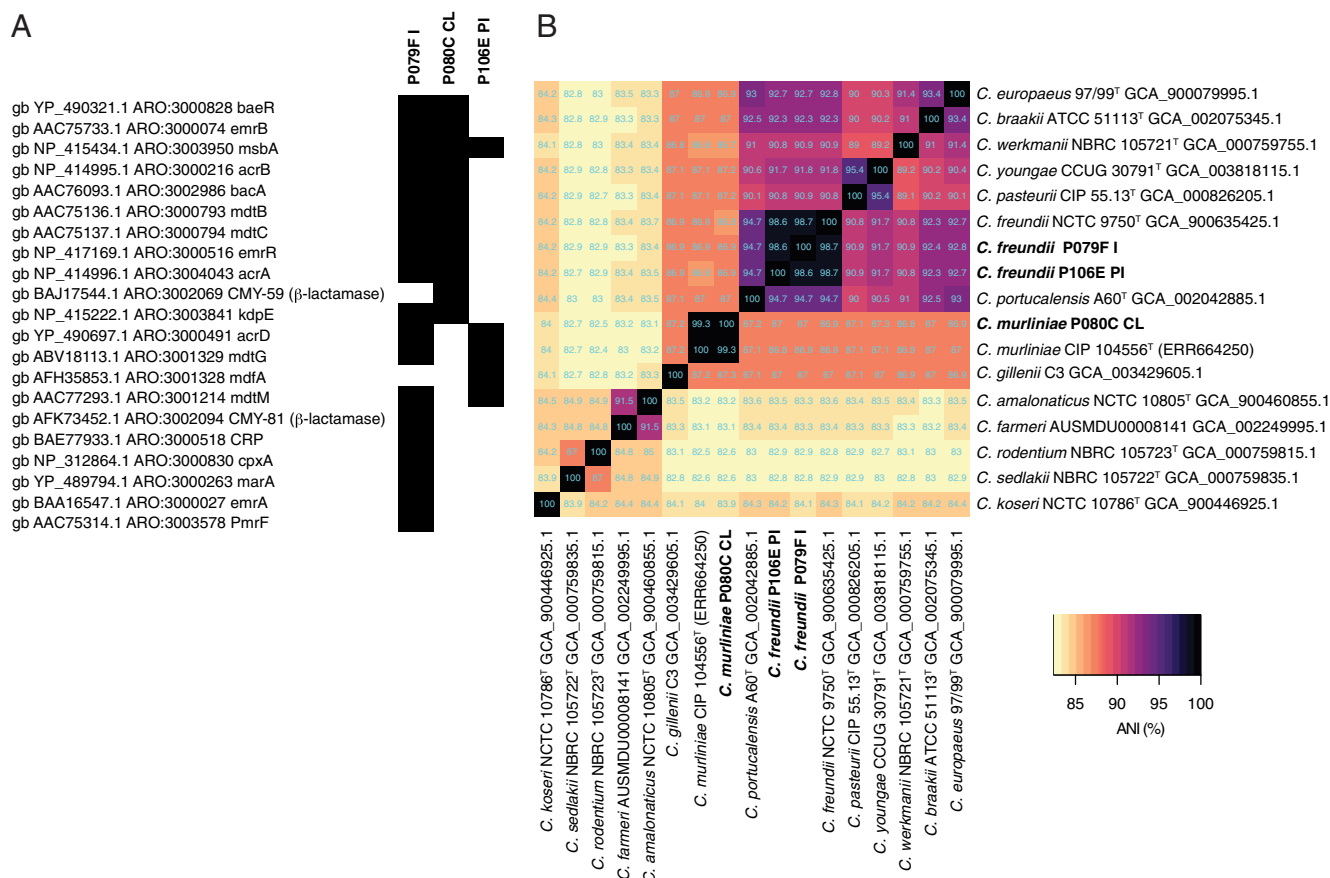


FIG 1 (A) Antibiotic resistance determinant homologs found in the genomes of the three *Citrobacter* strains recovered from the feces of preterm neonates. Antibiotic Resistance Ontology (ARO) annotations were retrieved from Comprehensive Antibiotic Resistance Database (CARD) matches, with only those homologs that gave a strict match with CARD reference sequences based on CARD-recommended bit score cutoffs (gene dependent) for BLASTP analyses included in the figure (black). White, no homologous match. (B) Heatmap showing ANI values obtained with FastANI (15) for representatives of the genus *Citrobacter* and the three neonate strains.

murlinae CIP 104556^T and is therefore a representative and first available draft genome sequence of this species (16–18).

Data availability. These whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession no. [QFTZ00000000](https://doi.org/10.1093/jis.0.000122) (P079F I), [QFVP00000000](https://doi.org/10.1093/jis.0.000122) (P080C CL), and [QFTQ00000000](https://doi.org/10.1093/jis.0.000122) (P106E PI). Raw data have been deposited under accession no. [SRR9048465](https://doi.org/10.1093/jis.0.000122), [SRR9048466](https://doi.org/10.1093/jis.0.000122), and [SRR9048464](https://doi.org/10.1093/jis.0.000122), respectively. The versions described in this paper are the first versions, QFTZ01000000, QFVP01000000, and QFTQ01000000, respectively.

ACKNOWLEDGMENTS

T.C.B. was funded by a University of Westminster Ph.D. studentship and by a Research Visit Grant from the Microbiology Society (grant RVG16/3). This work was funded via a Wellcome Trust Investigator Award to L.J.H. (100/974/C/13/Z), an Institute Strategic Program grant for Gut Health and Food Safety (BB/J004529/1), a BBSRC Institute Strategic Program Gut Microbes and Health grant (BB/R012490/1) and its constituent project BBS/E/F/000PR10353 (to L.J.H.), and by a BBSRC Norwich Research Park Bioscience Doctoral Training Grant (BB/M011216/1; supervisor, L.J.H.; student, C.A.-G.).

This work used the computing resources of the UK Medical Bioinformatics partnership (UK Med-Bio), which was supported by the Medical Research Council (grant MR/L01632X/1), and those of CLIMB (22).

This publication made use of the *Citrobacter freundii* MLST website (<https://pubmlst.org/cfreundii/>), sited at the University of Oxford (23) and accessed 16 April 2019.

L.H. is a member of the ESCMID Study Group for Host and Microbiota Interaction (https://www.escmid.org/research_projects/study_groups/host_and_microbiota_interaction/).

REFERENCES

- Clermont D, Motreff L, Passet V, Fernandez J-C, Bizet C, Brisse S. 2015. Multilocus sequence analysis of the genus *Citrobacter* and description of *Citrobacter pasteurii* sp. nov. *Int J Syst Evol Microbiol* 65:1486–1490. <https://doi.org/10.1093/jis.0.000122>.
- Bandyopadhyay T, Kumar A, Saili A, Randhawa VS. 2018. Distribution, antimicrobial resistance and predictors of mortality in neonatal sepsis. *J Neonatal Perinatal Med* 11:145–153. <https://doi.org/10.3233/NPM-1765>.
- Bae JY, Kang CK, Choi SJ, Lee E, Choe PG, Park WB, Kim NJ, Kim EC, Oh MD. 2018. Sudden deaths of neonates receiving intravenous infusion of lipid emulsion contaminated with *Citrobacter freundii*. *J Korean Med Sci* 33:e97. <https://doi.org/10.3346/jkms.2018.33.e97>.
- Obeng-Nkrumah N, Labi A-K, Addison NO, Labi JEM, Awuah-Mensah G. 2016. Trends in paediatric and adult bloodstream infections at a Ghanaian referral hospital: a retrospective study. *Ann Clin Microbiol Antimicrob* 15:49. <https://doi.org/10.1186/s12941-016-0163-z>.
- Stoesser N, Sheppard AE, Shakya M, Sthapit B, Thorson S, Giess A, Kelly D, Pollard AJ, Peto TE, Walker AS, Crook DW. 2015. Dynamics of MDR *Enterobacter cloacae* outbreaks in a neonatal unit in Nepal: insights using wider sampling frames and next-generation sequencing. *J Antimicrob Chemother* 70:1008–1015. <https://doi.org/10.1093/jac/dku521>.
- Arana DM, Ortega A, González-Barberá E, Lara N, Bautista V, Gómez-Ruiz D, Sáez D, Fernández-Romero S, Aracil B, Pérez-Vázquez M, Campos J, Oteo J, Spanish Antibiotic Resistance Surveillance Programme Collaborating Group. 2017. Carbapenem-resistant *Citrobacter* spp. isolated in Spain from 2013 to 2015 produced a variety of carbapenemases including VIM-1, OXA-48, KPC-2, NDM-1 and VIM-2. *J Antimicrob Chemother* 72:3283–3287. <https://doi.org/10.1093/jac/dkx325>.
- Chen Y, Brook TC, Alcon-Giner C, Clarke P, Hall LJ, Hoyle L. 2019. Draft genome sequence of *Raoultella ornithinolytica* P079F W, isolated from the feces of a preterm infant. *Microbiol Resource Announc*. <https://doi.org/10.1128/MRA.00493-19>.
- Kiu R, Caim S, Alcon-Giner C, Belteki G, Clarke P, Pickard D, Dougan G, Hall LJ. 2017. Preterm infant-associated *Clostridium tertium*, *Clostridium cadaveris*, and *Clostridium paraputrificum* strains: genomic and evolutionary insights. *Genome Biol Evol* 9:2707–2714. <https://doi.org/10.1093/gbe/evx210>.
- Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD. 2010. Evolution of MRSA during hospital transmission and intercontinental spread. *Science* 327:469–474. <https://doi.org/10.1126/science.1182395>.
- Segata N, Waldron L, Ballarini A, Narasimhan V, Jousson O, Huttenhower C. 2012. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat Methods* 9:811–814. <https://doi.org/10.1038/nmeth.2066>.
- Abbott JC. 2017. BugBuilder—an automated microbial genome assembly and analysis pipeline. *bioRxiv*. <https://doi.org/10.1101/148783>.
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FS, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Res* 45:D566–D573. <https://doi.org/10.1093/nar/gkw1004>.
- Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9:5114. <https://doi.org/10.1038/s41467-018-07641-9>.
- Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 68:461–466. <https://doi.org/10.1099/ijsem.0.002516>.
- Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P,

- Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijms.0.64483-0>.
18. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 106: 19126–19131. <https://doi.org/10.1073/pnas.0906412106>.
19. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
20. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 67: 1613–1617. <https://doi.org/10.1099/ijsem.0.001755>.
21. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 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>.
22. Connor TR, Loman NJ, Thompson S, Smith A, Southgate J, Poplawski R, Bull MJ, Richardson E, Ismail M, Thompson SE, Kitchen C, Guest M, Bakke M, Sheppard SK, Pallen MJ. 2016. CLIMB (the Cloud Infrastructure for Microbial Bioinformatics): an online resource for the medical microbiology community. *Microb Genom* 2:e000086. <https://doi.org/10.1099/mgen.0.000086>.
23. Jolley KA, Bray JE, Maiden M. 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 3:124. <https://doi.org/10.12688/wellcomeopenres.14826.1>.