Draft Genome Sequence of an *Escherichia coli* Strain Harboring *bla*$_{CTX-M-115}$, *bla*$_{CMY-2}$, Aminoglycoside, Tetracycline, and Sulfonamide Resistance Genes, Isolated from a Costa Rican Wastewater Treatment Plant

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ABSTRACT We report the draft genome sequence of the multidrug-resistant *Escherichia coli* strain PTA A1517-5, isolated from a wastewater treatment plant in Costa Rica. The genome consists of 4,927,375 bp with a GC content of 50.57% and a total of 4,853 genes. This strain harbors *bla*$_{CTX-M-115}$, *bla*$_{CMY-2}$, aminoglycoside, tetracycline, and sulfonamide resistance genes.

*E*scherichia coli is a well-known and frequently used indicator of fecal pollution. This bacterium has also been shown to be a reservoir of antimicrobial resistance (AMR) genes. Detection of *E. coli* harboring AMR genes could provide information on the occurrence and spread of antibiotic resistance in the environment (1–5).

E. coli strain PTA A1517-5 was isolated from a wastewater sample which was collected from the effluent of a domestic wastewater treatment plant (WWTP) located in Alajuela, Costa Rica. *E. coli* organisms were enumerated from the WWTP effluent using the most probable number (MPN) technique according to American Public Health Association (APHA) guidelines (6). Briefly, the wastewater sample was inoculated into lauryl tryptose broth (Oxoid) and incubated at 35.0°C for 48 h. All tubes testing positive after the incubation period were inoculated into EC-MUG broth (Oxoid). After a 24-h incubation period at 44.5°C, tubes with a positive reaction were inoculated onto MacConkey agar plates (Oxoid) and incubated at 35°C for 24 h. The *E. coli* strain was identified using biochemical (API20E; BioMérieux) and molecular (16S rRNA) methods (7).

The antibiotic susceptibility profile was assessed according to 2014 CLSI guidelines (15). The *E. coli* strain showed resistance to amoxicillin (AML), cephalothin (KF), cefazolin (KZ), cefotaxime (CTX), tetracycline (TE), gentamicin (CN), and trimethoprim-sulfamethoxazole (SXT).

After biochemical identification, a single colony of the *E. coli* strain was picked and further grown in Trypticase soy broth (Oxoid) at 35°C for 18 to 24 h. Genomic DNA was extracted from the *E. coli* strain using a DNeasy blood and tissue kit (Qiagen). DNA quality and quantity were measured using a NanoDrop instrument (Thermo Fisher, Waltham, MA, USA) and a Quantus fluorometer (Promega, Wisconsin, USA). A dilution of 0.2 ng/µl of genomic DNA was used to prepare libraries with a Nextera XT DNA library prep kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The library was sequenced on an Illumina MiSeq instrument using a paired-end (2 × 250-bp) protocol.

The paired-end reads' trimming quality was assessed using FastQC v0.11.5, and it was conducted with the seqtk toolkit (8) using the parameters q = 0.1 and L = 200. A total of 1,895,908 reads were obtained after trimming. Reads were assembled *de novo*
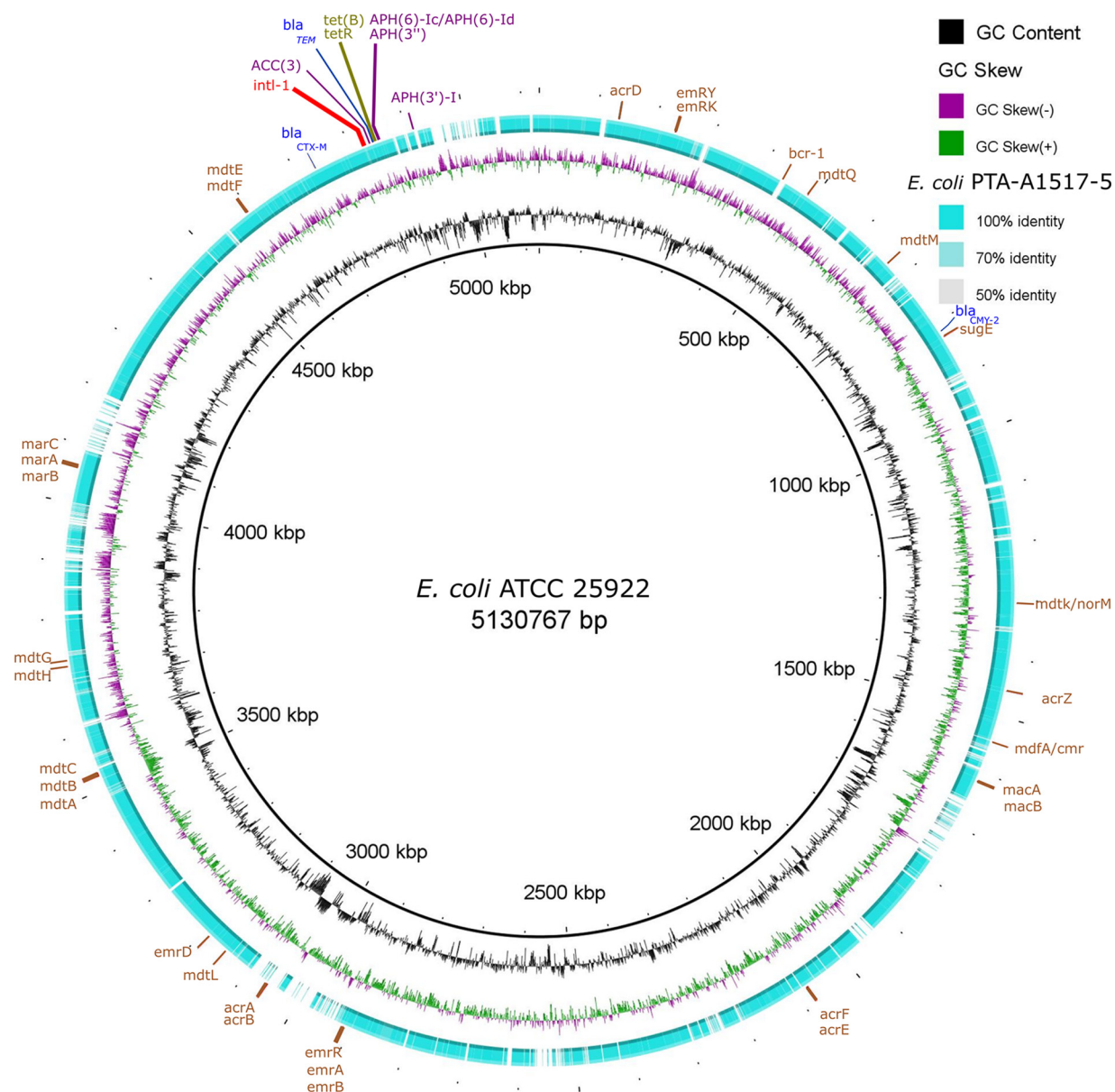
FIG 1 Genome alignment of *E. coli* strain PTA A1517-5 using the BLAST Ring Image Generator (BRIG) software (14). Multidrug efflux system genes are denoted in brown letters (*emrY, emrK, Bcr-1, mdtQ, mdtM, sugE, mdtK, norM, acrZ, mdfA/cmr, macA, macB, acrF, acrE, emrR, emrA, emrB, acrA, acrB, mdtL, emrD, mdtA, mdtB, mdtC, mdtH, mdtG, marA, marC, marB, mdtE,* and *mdtF*). Beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{CTX-M-115}$, and *bla*$_{CMY-2}$) are denoted in blue letters. Class 1 integron genes (*intI-1, dfrA12, gcuF, aadA2, qacEdelta1,* and *sul1*) are marked with red letters. Aminoglycoside resistance genes [*ACC(3), APH(3″)-I, APH(3″), APH(6)-Ic,* and *APH(6)-Id*] are denoted in purple letters, and tetracycline resistance genes [*tet(B)* and *tetR*] are marked with green letters. The genome of *E. coli* strain ATCC 25922 was used as the reference (GenBank accession no. CP009072).

using SPAdes v3.13.0 (9) with default settings and included a built-in BayesHammer read error correction tool. All contigs smaller than 500 bp were removed.

The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.8 (10).

The *E. coli* PTA A1517-5 draft genome sequence consists of 4,927,375 bp in 82 contigs with a GC content of 50.57%, an N_{50} value of 210,703 bp, a total of 4,853 genes, and a genome coverage of 31.2×.

AMR genes were queried in the ResFinder (11), PATRIC (12), and CARD databases (13) using default parameters.

The antibiotic resistance phenotype of strain PTA A1517-5 was consistent with the presence of *bla*$_{CTX-M-115}$, *bla*$_{CMY-2}$, aminoglycoside, tetracycline, and sulfonamide resistance genes.

BLAST Ring Image Generator (BRIG) (14) was used to visualize the coding sequence identity between the *E. coli* strain PTA A1517-5 and the reference *E. coli* strain ATCC 25922 (Fig. 1). Genes related to AMR are indicated in Fig. 1.

Data availability. This whole-genome shotgun sequencing project has been deposited in DDBJ/ENA/GenBank under the accession no. VMHG00000000. The version described in this paper is version VMHG02000000. The reads were deposited in the Sequence Read Archive (SRA) under accession no. PRJNA556251.

REFERENCES

1. Aslan A, Cole Z, Bhattacharya A, Oyibo O. 2018. Presence of antibiotic-resistant *Escherichia coli* in wastewater treatment plant effluents utilized as water reuse for irrigation. Water (Switzerland) 10. https://doi.org/10.3390/w10060805.

2. Karkman A, Pärnänen K, Larsson DGJ. 2019. Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. Nat Commun 10:1–8. https://doi.org/10.1038/s41467-018-07992-3.

3. Kappell AD, De Nies MS, Ahuja NH, Ledeboer NA, Newton RJ, Hristova KR. 2015. Detection of multi-drug resistant *Escherichia coli* in the urban waterways of Milwaukee, WI. Front Microbiol 6:1–12.

4. Watkinson AJ, Micalizzi GB, Graham GM, Bates JB, Costanzo SD. 2007. Antibiotic-resistant *Escherichia coli* in wastewaters, surface waters, and oysters from an urban riverine system. Appl Environ Microbiol 73: 5667–5670. https://doi.org/10.1128/AEM.00763-07.

5. Gomez-Alvarez V, Hoelle J. 2018. Draft genome sequences of antibiotic-resistant *Escherichia coli* isolates from U.S. wastewater treatment plants. Microbiol Resour Announc 5:18–19. https://doi.org/10.1128/MRA.00351-19.

6. APHA, AWWA, WEF. 2012. Standard methods for the examination of water and wastewater. 22nd ed. Part 9000 Microbiological Examination. American Public Health Association, Washington, DC.

7. Chacón JLM, Taylor CL, Valiente AC, Alvarado PI, Cortés BX. 2012. A DNA pooling based system to detect *Escherichia coli* virulence factors in fecal and wastewater samples. Braz J Microbiol 43:1319–1326. https://doi.org/10.1590/S1517-83822012000400012.

8. Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLoS One 11:e0163962. https://doi.org/10.1371/journal.pone.0163962.

9. 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.

10. 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.

11. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.

12. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res 45: D535–D542. https://doi.org/10.1093/nar/gkw1017.

13. 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 FSL, 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.

14. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. BMC Genomics 12:402. https://doi.org/10.1186/1471-2164-12-402.

15. Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing: 24th informational supplement, M100–S24. Clinical and Laboratory Standards Institute, Wayne, PA.