



# Whole-Genome Sequence Analysis of Multidrug-Resistant *Enterobacter hormaechei* Isolated from Imported Retail Shrimp

Nagaraju Indugu,<sup>b</sup> Laxmi Sharma,<sup>a</sup> Charlene R. Jackson,<sup>c</sup>  Prashant Singh<sup>a</sup>

<sup>a</sup>Department of Nutrition, Food and Exercise Sciences, Florida State University, Tallahassee, Florida, USA

<sup>b</sup>Department of Clinical Studies, Center for Animal Health and Productivity, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pennsylvania, USA

<sup>c</sup>Bacterial Epidemiology and Antimicrobial Resistance Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Athens, Georgia, USA

**ABSTRACT** Here, we announce the draft genome sequence of *Enterobacter hormaechei* 2B-MC1, isolated from a shrimp sample collected from a farmer's market in Atlanta, Georgia. The assembled genome sequence observed was 4,661,561 bp long with a G+C content of 55.3%. The isolate harbored *sul1*, *sul2*, *qnrA1*, *oqxB*, *dfrA23*, *bla<sub>ACT1</sub>*, *floR*, *fosA*, *tet(A)*, *aph(6)-I<sub>d</sub>*, and *aph(3'')-I<sub>b</sub>* antibiotic resistance genes.

We isolated and characterized a multidrug-resistant (MDR) *Enterobacter hormaechei* 2B-MC1 strain from raw farm-raised shrimp from Ecuador which was collected from a farmer's market in Atlanta, Georgia, on 30 March 2019. Thirty-one shrimp samples were screened for the presence of extended-spectrum  $\beta$ -lactam strains as previously described (1). The 110 isolates obtained were purified by streaking onto nutrient agar. The genus and species of isolates were identified after 16S rRNA gene sequencing using 27F and 511R primers (2). Isolates were screened for MDR strains using the disk diffusion assay (3). *E. hormaechei* 2B-MC1 was found to be resistant to multiple classes of antimicrobials and was selected for whole-genome sequencing. A pure culture of the strain was grown in tryptic soy broth, and DNA from the overnight culture was extracted using the DNeasy PowerFood microbial kit (Qiagen). The quality and quantity of the DNA were measured using a NanoDrop One spectrophotometer (Thermo Fisher). Libraries from the DNA sample were prepared and sequenced at a Florida State University (Florida, USA) molecular cloning facility. Libraries were sequenced using a MiSeq 2 microkit (Illumina) at 9 pM with 5% PhiX. Default parameters for all software were used during analyses unless otherwise specified. The raw Illumina sequences were quality filtered using Trimmomatic (v0.36) (4). The high-quality reads were *de novo* assembled with Velvet (1.2.10) (5) with a k-mer size of 51. We only considered contigs longer than 200 bp in computing. CheckM (v1.0.7) with "lineage\_wf" workflow (6) was used to estimate the completeness of the draft genome. The most closely related complete genomes of *E. hormaechei* 2B-MC1 were identified using Microbial Genomes Atlas MiGA online tool (7) with the NCBI prokaryotic genome database. The assemblies were annotated with the Prokka (v1.11) (8) annotation pipeline. Antimicrobial-resistant gene sequences were identified with NCBI AMRFinder (9). The analysis of harbored plasmid(s) within the draft genome were detected using the PLSDB database (10) with Mash software (11). The identified plasmids were further confirmed with BLAST alignment.

A total of 4,397,799 paired-end reads were sequenced. These raw sequences were trimmed and then quality filtered (about 8% of the reads were eliminated), resulting in approximately 363,705 reads. These high-quality reads were assembled into contigs. CheckM estimated the completeness of this genome as 99.8%. This assembly produced 160 contigs and an  $N_{50}$  value of 82,480 bp. This draft genome was 4,661,561 bp long with a G+C content of 55.3%. Genome annotation with Prokka identified 4,336 open

**Citation** Indugu N, Sharma L, Jackson CR, Singh P. 2020. Whole-genome sequence analysis of multidrug-resistant *Enterobacter hormaechei* isolated from imported retail shrimp. Microbiol Resour Announc 9:e01103-20. <https://doi.org/10.1128/MRA.01103-20>.

**Editor** Frank J. Stewart, Georgia Institute of Technology

**Copyright** © 2020 Indugu 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 Prashant Singh, [psingh2@fsu.edu](mailto:psingh2@fsu.edu).

**Received** 6 October 2020

**Accepted** 10 November 2020

**Published** 10 December 2020

reading frames and 36 RNAs (1 rRNA, 34 tRNAs, and 1 transfer-messenger RNA [tmRNA]). A total of 1,567 genes were associated with KEGG pathways. The closest relatives of *E. hormaechei* 2B-MC1 found by MiGA in the database were *Enterobacter* sp. strain CRENT-193 (GenBank accession number [NZ\\_CP024812.1](#)) and *E. hormaechei* ([NZ\\_CP030076](#)) with an average nucleotide identity of 99.19%. AMRFinder identified genes and mutations that confer resistance to sulfonamides (*sul1* and *sul2*), quinolones (*qnrA1*), phenicol/quinolones (*oqxB*), trimethoprim (*dfrA23*), beta-lactamases (*bla<sub>ACT</sub>*), phenicol (*floR*), fosfomycin (*fosA*), tetracycline [*tet(A)*], and aminoglycosides [*aph(6)-Ia* and *aph(3'')-Ib*]. The following six putative plasmids were present in the draft genome: pESBL176 ([MT230180.1](#)), pESBL31 ([MT230288.1](#), [MT230289.1](#), [MT230292.1](#), and [MT230305.1](#)), pESBL87 ([MT230381.1](#)), pESBL96 ([MT230424.1](#) and [MT230426.1](#)), unnamed4 plasmid ([NZ\\_CP020513.1](#)), and pKP2442\_7c331 ([NZ\\_KX434882.1](#)).

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [JACVEK000000000](#). The version described in this paper is the first version, [JACVEK010000000](#). Raw Illumina data have been deposited in the NCBI Sequence Read Archive with the accession number [SRR12586817](#) under the BioProject accession number [PRJNA661375](#).

## ACKNOWLEDGMENT

This study was funded by Florida State University startup funds.

## REFERENCES

- Hutchinson H, Finney S, Muñoz-Vargas L, Feicht S, Masterson M, Habing G. 2017. Prevalence and transmission of antimicrobial resistance in a vertically integrated veal calf production system. *Foodborne Pathog Dis* 14:711–718. <https://doi.org/10.1089/fpd.2017.2310>.
- Liu A-C, Chou C-Y, Chen L-L, Kuo C-H. 2015. Bacterial community dynamics in a swine wastewater anaerobic reactor revealed by 16S rDNA sequence analysis. *J Biotechnol* 194:124–131. <https://doi.org/10.1016/j.jbiotec.2014.11.026>.
- Clinical and Laboratory Standards Institute. 2015. Performance standards for antimicrobial disk susceptibility tests; approved standard—12th edition. Clinical and Laboratory Standards Institute, Wayne, PA.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
- 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>.
- Rodriguez-R LM, Gunturu S, Harvey WT, Rosselló-Mora R, Tiedje JM, Cole JR, Konstantinidis KT. 2018. The Microbial Genomes Atlas (MiGA) web-server: taxonomic and gene diversity analysis of Archaea and Bacteria at the whole genome level. *Nucleic Acids Res* 46:W282–W288. <https://doi.org/10.1093/nar/gky467>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu C-H, McDermott PF, Tadesse DA, Morales C, Simmons M, Tillman G, Wasilenko J, Folster JP, Klimke W. 2019. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. *Antimicrob Agents Chemother* 63:e00483-19. <https://doi.org/10.1128/AAC.00483-19>.
- Galata V, Fehlmann T, Backes C, Keller A. 2019. PLSDB: a resource of complete bacterial plasmids. *Nucleic Acids Res* 47:D195–D202. <https://doi.org/10.1093/nar/gky1050>.
- Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, Phillippy AM. 2016. Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol* 17:132. <https://doi.org/10.1186/s13059-016-0997-x>.