



Complete Genome Sequence of a Ciprofloxacin-Resistant *Salmonella enterica* subsp. *enterica* Serovar Kentucky Sequence Type 198 Strain, PU131, Isolated from a Human Patient in Washington State

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ABSTRACT Strains of the ciprofloxacin-resistant (Cip^r) *Salmonella enterica* subsp. *enterica* serovar Kentucky sequence type 198 (ST198) have rapidly and extensively disseminated globally to become a major food safety and public health concern. Here, we report the complete genome sequence of a Cip^r *S. Kentucky* ST198 strain, PU131, isolated from a human patient in Washington State (USA).

The ciprofloxacin-resistant (Cip^r) *Salmonella enterica* subsp. *enterica* serovar Kentucky sequence type 198 (ST198) has emerged as a global human pathogen. Human illnesses caused by this pathogen in North America and Europe are associated with history of travel to Africa, Southeast Asia, and the Middle East, where this pathogen is established in poultry (1–7). Cip^r *S. Kentucky* ST198 is also established in poultry in France, Poland, and other European countries and represents a significant risk to the public health and food safety (1, 2). Recently, a genetically distinct lineage of *S. Kentucky* ST198 susceptible to ciprofloxacin was reported in dairy cattle in the United States (8). Here, we report the first complete genome sequence of Cip^r *S. Kentucky* ST198 strain PU131, isolated in 2013 from a human patient in Washington State. An individual colony of *S. Kentucky* strain PU131 was grown overnight at 37°C in LB broth (Difco). DNA was extracted using a Qiagen DNeasy kit (Qiagen). The PacBio library was constructed following the manufacturer's protocol and size selected using BluePippin, with an average fragment size of 19 kb (range, 12.3 to 35 kb). Sequencing was performed using single-molecule real-time (SMRT) cells in an RS II sequencer (Molecular Biology and Genomics Core, Washington State University, Pullman, WA). A total of 89,926 reads (179.4× coverage), with mean read size of 12,595 bp and N_{50} value of 17,462 bp, were assembled using the Hierarchical Genome Assembly Process 2 (HGAP 2) workflow to obtain a 4,900,326-bp circularized consensus sequence, with 52.2% GC content. The serovar designation and multilocus sequence type (MLST) were confirmed *in silico* using EnteroBase (<https://enterobase.warwick.ac.uk/species/index/senterica>) and SISTR (9), respectively. The NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok) predicted 4,995 genes, including 4,873 coding sequences (CDSs), 22 rRNAs (10), 85 tRNAs (11), 14 noncoding RNAs (ncRNAs) (12), and 1 transfer-messenger RNA (tmRNA) (13). Additionally, 7 riboswitches (14), 2 clustered regularly interspaced short palindromic repeat (CRISPR) arrays (15) and 198 pseudogenes were identified. No plasmids were detected using PlasmidFinder version 1.3 (16). *S. Kentucky* strain PU131 was resistant to ampicillin, amoxicillin-clavulanic acid, chloramphenicol, tetracycline, sulfamethoxazole-trimethoprim, streptomycin, kanamycin, nalidixic acid, and ciprofloxacin. Quinolone resistance-determining regions (QRDRs) of the target genes *gyrA*, *gyrB*, *parC*, and *parE* showed 2 mutations in *gyrA* (Ser83Phe and

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Asp87Gly) and 3 mutations in *parC* (Ser80Ile, Thr57Ser, and Thr255Ser). The two *gyrA* mutations together with one *parC* mutation (Ser80Ile) suggest high-level fluoroquinolone resistance (17). Corresponding resistance genes [*bla*_{TEM1-B}, *cmlA1*, *tet(A)*, *sul1*, *sul3*, *dfrA12*, *aadA1*, *aadA2*, *aph(3')-Ia*, and *mph(A)*] were identified through ResFinder version 3.0 (18). PHAST analysis showed the presence of 3 intact, 3 incomplete, and 2 questionable prophage elements (19). *S. Kentucky* strain PU131 carries a 25.9-kb *Salmonella* genomic island-1 (SGI-1) inserted at the *trmE-yidY* locus with 23 open reading frames (C1D15_24950 to C1D15_24845). Comparison with SGI-1K (GenBank accession number AY463797) using progressiveMAUVE (20) and multigene BLAST (21) revealed that an ~24-kb region corresponding to SGI-1K ORFs *resG-S044* is deleted from strain PU131, with multiple insertions elsewhere in the genome. This complete genome sequence will aid in developing improved detection/subtyping methods for epidemiological source tracing and to achieve a better understanding of the pathogenicity and antimicrobial resistance of this emerging pathogen.

Accession number(s). The genome sequence is deposited in NCBI GenBank (BioProject number PRJNA428776, accession number CP026327). The version described is the first version.

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REFERENCES

1. Le Hello S, Bekhit A, Granier S, Barua H, Beutlich J, Zajac M, Münch S, Sintchenko V, Bouchrif B, Fashae K, Pinsard J-L, Sontag L, Fabre L, Garnier M, Guibert V, Howard P, Hendriksen R, Christensen J, Biswas P, Cloeckert A, Rabsch W, Wasyl D, Doublet B, Weill F-X. 2013. The global establishment of a highly-fluoroquinolone resistant *Salmonella enterica* serotype Kentucky ST198 strain. *Front Microbiol* 4:395. <https://doi.org/10.3389/fmicb.2013.00395>.
2. Le Hello S, Hendriksen RS, Doublet B, Fisher I, Nielsen EM, Whichard JM, Bouchrif B, Fashae K, Granier SA, Silva NJD, Cloeckert A, Threlfall EJ, Angulo FJ, Aarestrup FM, Wain J, Weill FX. 2011. International spread of an epidemic population of *Salmonella enterica* serotype Kentucky ST198 resistant to ciprofloxacin. *J Infect Dis* 204:675–684. <https://doi.org/10.1093/infdis/jir409>.
3. Mulvey MR, Boyd DA, Finley R, Fakharuddin K, Langner S, Allen V, Ang L, Bekal S, El Bailey S, Haldane D, Hoang L, Horsman G, Louis M, Robberts L, Wylie J. 2013. Ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky in Canada. *Emerg Infect Dis* 19:999–1001. <https://doi.org/10.3201/eid1906.121351>.
4. Raufu IA, Fashae K, Ameh JA, Ambali A, Ogunsola FT, Coker AO, Hendriksen RS. 2014. Persistence of fluoroquinolone-resistant *Salmonella enterica* serovar Kentucky from poultry and poultry sources in Nigeria. *J Infect Dev Ctries* 8:384–388. <https://doi.org/10.3855/jidc.3495>.
5. Shah DH, Paul NC, Sischo WC, Crespo R, Guard J. 2017. Population dynamics and antimicrobial resistance of the most prevalent poultry-associated *Salmonella* serotypes. *Poult Sci* 96:687–702. <https://doi.org/10.3382/ps/pew342>.
6. Westrell T, Monnet DL, Gossner C, Heuer O, Takkinen J. 2014. Drug-resistant *Salmonella enterica* serotype Kentucky in Europe. *Lancet Infect Dis* 14:270–271. [https://doi.org/10.1016/S1473-3099\(14\)70703-0](https://doi.org/10.1016/S1473-3099(14)70703-0).
7. Rickert-Hartman R, Folster JP. 2014. Ciprofloxacin-resistant *Salmonella enterica* serotype Kentucky sequence type 198. *Emerg Infect Dis* 20: 910–911. <https://doi.org/10.3201/eid2005.131575>.
8. Haley BJ, Kim SW, Pettengill J, Luo Y, Karns JS, Van Kessel JAS. 2016. Genomic and evolutionary analysis of two *Salmonella enterica* serovar Kentucky sequence types isolated from bovine and poultry sources in North America. *PLoS One* 11:e0161225. <https://doi.org/10.1371/journal.pone.0161225>.
9. Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VPJ, Nash JHE, Taboada EN. 2016. The *Salmonella In Silico* Typing Resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft *Salmonella* genome assemblies. *PLoS One* 11:e0147101. <https://doi.org/10.1371/journal.pone.0147101>.
10. Pei AY, Oberdorf WE, Nossa CW, Agarwal A, Chokshi P, Gerz EA, Jin ZD, Lee P, Yang LY, Poles M, Brown SM, Sotero S, DeSantis T, Brodie E, Nelson K, Pei ZH. 2010. Diversity of 16S rRNA genes within individual prokaryotic genomes. *Appl Environ Microbiol* 76:5333–3897. <https://doi.org/10.1128/AEM.01365-10>.
11. Raina M, Ibba M. 2014. tRNAs as regulators of biological processes. *Front Genet* 5:171. <https://doi.org/10.3389/fgene.2014.00171>.
12. Repoila F, Darfeuille F. 2009. Small regulatory non-coding RNAs in bacteria: physiology and mechanistic aspects. *Biol Cell* 101:117–131. <https://doi.org/10.1042/BC20070137>.
13. Himeno H, Kurita D, Muto A. 2014. tmRNA-mediated trans-translation as the major ribosome rescue system in a bacterial cell. *Front Genet* 5:66. <https://doi.org/10.3389/fgene.2014.00066>.
14. Edwards AL, Batey RT. 2010. Riboswitches: a common RNA regulatory element. *Nat Educ* 3:9.
15. Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res* 35:W52–W57. <https://doi.org/10.1093/nar/gkm360>.
16. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, 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>.
17. Baucheron S, Le Hello S, Doublet B, Giraud E, Weil FX, Cloeckert A. 2013. *ramR* mutations affecting fluoroquinolone susceptibility in epidemic multidrug-resistant *Salmonella enterica* serovar Kentucky ST198. *Front Microbiol* 4:213. <https://doi.org/10.3389/fmicb.2013.00213>.
18. 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>.
19. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39:W347–W352. <https://doi.org/10.1093/nar/gkr485>.
20. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e11147. <https://doi.org/10.1371/journal.pone.0011147>.
21. Medema MH, Takano E, Breitling R. 2013. Detecting sequence homology at the gene cluster level with MultiGeneBlast. *Mol Biol Evol* 30:1218–1223. <https://doi.org/10.1093/molbev/mst025>.