



Complete Genome Sequences of African *Salmonella enterica* Serovar Enteritidis Clinical Isolates Associated with Bloodstream Infection

Blanca M. Perez-Sepulveda,^a Alexander V. Predeus,^a Wai Yee Fong,^a Christopher M. Parry,^a John Cheesbrough,^{a,b} Paul Wigley,^a Nicholas A. Feasey,^{c,d} Jay C. D. Hinton^a

^aInstitute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, United Kingdom

^bInstitute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom

^cLiverpool School of Tropical Medicine, Liverpool, United Kingdom

^dMalawi-Liverpool-Wellcome Programme, Blantyre, Malawi

ABSTRACT We report the complete genome sequencing and annotation of four *Salmonella enterica* serovar Enteritidis isolates, two that are representative of the Central/Eastern African clade (CP255 and D7795) and two of the Global Epidemic clade (A1636 and P125109).

Salmonella enterica serovar Enteritidis typically causes gastroenteritis and is responsible for a global epidemic linked to poultry and egg production. Over the recent decades, *S. Enteritidis* has become a leading cause of invasive nontyphoidal *Salmonella* (iNTS) disease in sub-Saharan Africa (1, 2), and novel clades of this serovar have been isolated from individuals with bloodstream infection (3). In contrast to the Global Epidemic clade, these novel African clades are associated with high mortality in immunocompromised individuals and are typically multidrug resistant (MDR), representing an important public health challenge (4).

We used long-read sequencing to investigate the genome sequences of two representative *S. Enteritidis* strains of the Central/Eastern African clade (CP255 and D7795) and two of the Global Epidemic clade (P125109 and A1636). CP255 was isolated in the Democratic Republic of Congo (then Zaire) in 1991, from the blood of a child at the Institut Médical Evangélique, Kimpese, and is phenotypically MDR (amoxicillin, tetracycline, chloramphenicol, and streptomycin resistant) (5, 6). D7795 (pediatric patient/MDR) and A1636 (adult patient/fully susceptible) were isolated in 1998 and 2000, respectively, in Blantyre, Malawi (3). *S. Enteritidis* P125109, a UK PT4 isolate from 1988, was used as a reference (7–10).

A single colony of each isolate was grown for 16 h in 5 ml of Lennox medium at 37°C. Total DNA was extracted using the Quick-DNA universal kit (Zymo; catalog number D4069). DNA integrity was verified by 0.5% agarose gel electrophoresis at 90 V for 1.5 h. DNA purity/concentration were measured with a DeNovix DS-11FX spectrophotometer/fluorometer and Qubit double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit. Long-read sequencing was performed by the Centre for Genomic Research (University of Liverpool, UK) in a PacBio single-molecule real-time (SMRT) cell (P6/C4 chemistry) using SMRTbell Template v1.0 (Pacific Biosciences; catalog number 100-259-100) library preparation with g-TUBE (Covaris) fragmentation and size selection of 15 to 50 kb with 0.75% agarose cassette (BluePippin; catalog number BMF7510). Illumina HiSeq sequencing was performed as part of the 10KSG project (11) and by MicrobesNG (UK) using the Nextera XT library prep kit (Illumina, USA) with modifications (2 ng DNA and 1 min PCR elongation) and 250-bp paired-end protocol. The reads were adapter trimmed using Trimmomatic v0.30, with a sliding window quality cutoff of Q15 (12).

Citation Perez-Sepulveda BM, Predeus AV, Fong WY, Parry CM, Cheesbrough J, Wigley P, Feasey NA, Hinton JCD. 2021. Complete genome sequences of African *Salmonella enterica* serovar Enteritidis clinical isolates associated with bloodstream infection. *Microbiol Resour Announc* 10:e01452-20. <https://doi.org/10.1128/MRA.01452-20>.

Editor Vincent Bruno, University of Maryland School of Medicine

Copyright © 2021 Perez-Sepulveda 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 Jay C. D. Hinton, jay.hinton@liverpool.ac.uk.

Received 17 December 2020

Accepted 21 February 2021

Published 25 March 2021

TABLE 1 Characteristics and accession numbers of the genome sequences of four *S. Enteritidis* isolates

Isolate	Yr	GenBank accession no.	SRA accession no. for:		No. of Illumina reads (2 × 250 bp)	Illumina coverage (x)	No. of PacBio reads	PacBio read N ₅₀ (bp)	G+C content (%)	Genome size (bp)	Total no. of genes	Data for virulence plasmid:		Data for other plasmids:		No. of SNPs for isolate: ^b	
			Illumina reads	PacBio reads								Name ^a	Size (kb)	Name ^a	Size (kb)	P125109	D7795
CP255	1991	GCA_015240995.1	SRR12953596	SRR12953597	296,730	30.6	40,000	20,451	52.30	4,840,946	4,729	pSEN-DRC	96	pRSF1010	8.7	972	61
D7795	2000	GCA_015240855.1	SRR12953602	SRR12953603	813,469	83.5	103,762	16,718	52.30	4,869,504	4,777	pSEN-BT (3)	116	pRGI00316	4.9	1,017	0
A1636	1998	GCA_015241115.1	SRR12953598	SRR12953599	3,859,080	406.4	97,435	16,873	52.20	4,748,456	4,608	pSENV	59	pSE-GC	3.2	46	1,022
P125109	1988	GCA_015240635.1	SRR12953600	SRR12953601	587,289	61.9	92,761	17,272	52.20	4,745,224	4,606	pSENV	59		0	0	1,022

^a Numbers in parentheses indicate references.

^b SNPs, single nucleotide polymorphisms identified in whole-genome-based sequence comparison against either *S. Enteritidis* P125109 or D7795.

Using Filtlong v0.2.0 (<https://github.com/rrwick/Filtlong>) and Illumina reads as a reference, we selected a subset of raw PacBio reads with the best quality and length to yield an approximate 100× coverage for each genome sequence. Selected long and short reads were assembled using Unicycler v0.4.4 in hybrid mode (13). The genome sequences were initially annotated using Prokka v1.13.7 (14) through Bacpipe v0.6 (<https://github.com/apredeus/multi-bacpipe>), automatically reannotated by GenBank with PGAP (15), and rotated to the origin at the *thrLABC* operon. Variant calling was done with Snippy v4.3.6 (<https://github.com/tseemann/snippy>) in contig mode.

Genome comparison revealed genomic degradation and differences in accessory genomes (Table 1). P125109 and A1636 carried the virulence plasmid pSENV, whereas D7795 carried pSEN-BT (3) and CP255 carried pSEN-DRC, which both had a pSENV backbone with multidrug resistance-encoding genes. Other plasmids were identified in A1636, D7795, and CP255. The prophage repertoire of A1636 resembled P125109 (7). Both D7795 and CP255 lacked Φ SE20 and instead carried P88-like and Fels2-like prophages.

Data availability. The annotated complete genome assemblies of *S. Enteritidis* CP255, D7795, A1636, and P125109 have been deposited at NCBI GenBank. The BioProject accession number is [PRJNA671837](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA671837), and the individual BioSample accession numbers are [SAMN16552338](https://www.ncbi.nlm.nih.gov/biosample/SAMN16552338) (A1636), [SAMN16552337](https://www.ncbi.nlm.nih.gov/biosample/SAMN16552337) (CP255), [SAMN16552336](https://www.ncbi.nlm.nih.gov/biosample/SAMN16552336) (D7795), and [SAMN16552335](https://www.ncbi.nlm.nih.gov/biosample/SAMN16552335) (P125109). The Bacpipe annotation pipeline can be accessed at <https://github.com/apredeus/multi-bacpipe>.

ACKNOWLEDGMENTS

We are grateful to present and former members of the Hinton laboratory for helpful discussions, particularly Rocío Canals and Siân Owen for their expert advice. We are indebted to Paul Barrow for kindly sharing the *S. Enteritidis* strain P125109.

This project was supported by the Wellcome Trust Senior Investigator Award (106914/Z/15/Z) to Jay C. D. Hinton. The MLW bacteremia service is funded by Wellcome Africa and Asia program grant 206545/Z/17/Z.

REFERENCES

- Reddy EA, Shaw AV, Crump JA. 2010. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 10:417–432. [https://doi.org/10.1016/S1473-3099\(10\)70072-4](https://doi.org/10.1016/S1473-3099(10)70072-4).
- Feasey NA, Masesa C, Jassi C, Faragher EB, Mallewa J, Mallewa M, MacLennan CA, Msefula C, Heyderman RS, Gordon MA. 2015. Three epidemics of invasive multidrug-resistant *Salmonella* bloodstream infection in Blantyre, Malawi, 1998–2014. *Clin Infect Dis* 61:S363–S371. <https://doi.org/10.1093/cid/civ691>.
- Feasey NA, Hadfield J, Keddy KH, Dallman TJ, Jacobs J, Deng X, Wigley P, Barquist L, Langridge GC, Feltwell T, Harris SR, Mather AE, Fookes M, Aslett M, Msefula C, Kariuki S, MacLennan CA, Onsare RS, Weill F-X, Le Hello S, Smith AM, McClelland M, Desai P, Parry CM, Cheesbrough J, French N, Campos J, Chabalgoity JA, Betancor L, Hopkins KL, Nair S, Humphrey TJ, Lunguya O, Cogan TA, Tapia MD, Sow SO, Tennant SM, Bornstein K, Levine MM, Lacharme-Lora L, Everett DB, Kingsley RA, Parkhill J, Heyderman RS, Dougan G, Gordon MA, Thomson NR. 2016. Distinct *Salmonella* Enteritidis lineages associated with enterocolitis in high-income settings and invasive disease in low-income settings. *Nat Genet* 48:1211–1217. <https://doi.org/10.1038/ng.3644>.
- Uche IV, MacLennan CA, Saul A. 2017. A systematic review of the incidence, risk factors and case fatality rates of invasive nontyphoidal *Salmonella* (iNTS) disease in Africa (1966 to 2014). *PLoS Negl Trop Dis* 11:e0005118. <https://doi.org/10.1371/journal.pntd.0005118>.
- Green SDR, Cheesbrough JS. 1993. *Salmonella* bacteraemia among young children at a rural hospital in western Zaire. *Ann Trop Paediatr* 13:45–53. <https://doi.org/10.1080/02724936.1993.11747624>.
- Cheesbrough JS, Taxman BC, Green SDR, Mewa FI, Numbi A. 1997. Clinical definition for invasive *Salmonella* infection in African children. *Pediatr Infect Dis J* 16:277–283. <https://doi.org/10.1097/00006454-199703000-00005>.
- Thomson NR, Clayton DJ, Windhorst D, Vernikos G, Davidson S, Churcher C, Quail MA, Stevens M, Jones MA, Watson M, Barron A, Layton A, Pickard D, Kingsley RA, Bignell A, Clark L, Harris B, Ormond D, Abdellah Z, Brooks K, Cherevach I, Chillingworth T, Woodward J, Norberczak H, Lord A, Arrowsmith C, Jagels K, Moule S, Mungall K, Sanders M, Whitehead S, Chabalgoity JA, Maskell D, Humphrey T, Roberts M, Barrow PA, Dougan G, Parkhill J. 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Res* 18:1624–1637. <https://doi.org/10.1101/gr.077404.108>.
- Silva CA, Blondel CJ, Quezada CP, Porwollik S, Andrews-Polymenis HL, Toro CS, Zaldivar M, Contreras I, McClelland M, Santiviago CA. 2012. Infection of mice by *Salmonella enterica* serovar Enteritidis involves additional genes that are absent in the genome of serovar Typhimurium. *Infect Immun* 80:839–849. <https://doi.org/10.1128/IAI.05497-11>.
- Barrow PA. 1991. Experimental infection of chickens with *Salmonella* enteritidis. *Avian Pathol* 20:145–153. <https://doi.org/10.1080/03079459108418749>.
- Barrow PA, Lovell MA. 1991. Experimental infection of egg-laying hens with *Salmonella* enteritidis phage type 4. *Avian Pathol* 20:335–348. <https://doi.org/10.1080/03079459108418769>.
- Perez-Sepulveda BM, Heavens D, Pulford CV, Predeus AV, Low R, Webster H, Schudoma C, Rowe W, Lipscombe J, Watkins C, Kumwenda B, Shearer N, Costigan K, Baker KS, Feasey NA, Hinton JCD, Hall N, The 10KSG Consortium. 2020. An accessible, efficient and global approach for the large-scale sequencing of bacterial genomes. *bioRxiv* <https://doi.org/10.1101/2020.07.22.200840>.
- 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>.
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595. <https://doi.org/10.1371/journal.pcbi.1005595>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- 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>.
16. Kröger C, Dillon SC, Cameron ADS, Papenfort K, Sivasankaran SK, Hokamp K, Chao Y, Sittka A, Hebrard M, Handler K, Colgan A, Leekitcharoenphon P, Langridge GC, Lohan AJ, Loftus B, Lucchini S, Ussery DW, Dorman CJ, Thomson NR, Vogel J, Hinton JCD. 2012. The transcriptional landscape and small RNAs of *Salmonella enterica* serovar Typhimurium. *Proc Natl Acad Sci U S A* 109:E1277–E1286. <https://doi.org/10.1073/pnas.1201061109>.
17. Jørgensen TS, Xu Z, Hansen MA, Sørensen SJ, Hansen LH. 2014. Hundreds of circular novel plasmids and DNA elements identified in a rat cecum metatranscriptome. *PLoS One* 9:e87924. <https://doi.org/10.1371/journal.pone.0087924>.