AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Complete Genome Sequence of Multidrug-Resistant *Salmonella enterica* Serovar I 4,[5],12:i:− 2015 U.S. Pork Outbreak Isolate USDA15WA-1

**Bradley L. Bearson,[a] Julian M. Trachsel,[b] Devin B. Holman,[b] Brian W. Brunelle,[b] Sathesh K. Sivasankaran,[b,c] Mustafa Simmons,[d] Jamie Wasilenko,[d] Glenn Tillman,[d] John J. Johnston,[e] Shawn M. D. Bearson[b]**

[a]National Laboratory for Agriculture and the Environment, Agroecosystems Management Research Unit, USDA-ARS, Ames, Iowa, USA
[b]National Animal Disease Center, Food Safety and Enteric Pathogens, USDA-ARS, Ames, Iowa, USA
[c]Genome Informatics Facility, Iowa State University, Ames, Iowa, USA
[d]OPHS Eastern Laboratory, USDA, FSIS, Athens, Georgia, USA
[e]Office of Public Health Science, USDA, FSIS, Fort Collins, Colorado, USA

**ABSTRACT** The genome of a multidrug-resistant (MDR) *Salmonella enterica* subsp. *enterica* serovar I 4,[5],12:i:− isolate from the 2015 U.S. pork outbreak was sequenced. The complete nucleotide sequence of USDA15WA-1 is 5,031,277 bp, including *Salmonella* genomic island 4 encoding tolerance to multiple metals and an MDR module inserted in the *fljB* region.

Nontyphoidal *Salmonella* spp. are a leading bacterial causative agent of human foodborne illness (1); however, many of the >2,600 serovars of *Salmonella* frequently colonize food animals (poultry, cattle, and swine) without causing clinical disease (2). In the United States, *Salmonella enterica* subsp. *enterica* serovar I 4,[5],12:i:− has emerged as the fourth most commonly isolated *Salmonella* serovar and the most prevalent multidrug-resistant (MDR) *Salmonella* serovar (68% of *Salmonella* I 4,[5],12:i:− strains are resistant to ≥3 CLSI antimicrobial classes) (3). In 2015, a multistate outbreak of MDR serovar I 4,[5],12:i:− was associated with pork products from Washington State; the *Salmonella* outbreak isolates were resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline (R-type ASSuT) (4). As part of this outbreak investigation, the USDA Food Safety and Inspection Service (FSIS) collected cecal samples from pigs postslaughter, as described in FSIS directive 10,100.1 (5). *Salmonella* spp. were isolated from cecal samples, as described in the USDA microbiology guidebook (6), modified to use 10 g of cecal content diluted 1:10 in buffered peptone water as the primary enrichment. In this report, we describe the complete genome sequence (NCBI accession number CP040686) of one of the recovered isolates, USDA15WA-1 (alternate identifier, FSIS1503788), of *Salmonella* serovar I 4,[5],12:i:−, associated with the pork outbreak.

Genomic DNA from strain USDA15WA-1 was extracted from an overnight culture grown in LB broth at 37°C using the Roche High Pure PCR template preparation kit, according to the manufacturer's instructions. The DNA was submitted to the Yale Center for Genome Analysis for single-molecule real-time (SMRT) sequencing on a PacBio RS II platform (Pacific Biosciences). Unless otherwise specified, default parameters were used for software analysis. Canu (1.4.1) was used for *de novo* assembly of the PacBio raw reads (7). The Canu output provided a single contig of an expected size, suggesting a complete genome. Trimming and quality filtering of Illumina MiSeq reads (SRA accession number SRR2421550) was performed using Trimmomatic (0.36) (8). The leading and trailing 3 bp were removed, as were any sequences with a quality score of <15 over a 4-bp sliding window. Reads with a minimum length of 50 bp were retained. The trimmed and paired MiSeq reads were aligned to the PacBio assembly with

**Citation** Bearson BL, Trachsel JM, Holman DB, Brunelle BW, Sivasankaran SK, Simmons M, Wasilenko J, Tillman G, Johnston JJ, Bearson SMD. 2019. Complete genome sequence of multidrug-resistant *Salmonella enterica* serovar I 4,[5],12:i:− 2015 U.S. pork outbreak isolate USDA15WA-1. Microbiol Resour Announc 8:e00791-19. https://doi.org/10.1128/MRA.00791-19.

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



Address correspondence to Bradley L. Bearson, brad.bearson@usda.gov.

**Received** 14 July 2019
**Accepted** 16 September 2019
**Published** 3 October 2019

**TABLE 1** Information used to assemble the genome of USDA15WA-1

| SRA accession no. | Library name | Read type | No. of reads | Avg read length (bp) |
|---|---|---|---|---|
| SRR2421550 | FSIS1503788 Nextera XT shotgun library | PE | 1,486,864 | 108.263 |
| SRR9119442 | USDA15WA-1_pacbio2 | Single | 151,170 | 7,863.24 |
| SRR9119443 | USDA15WA-1_pacbio1 | Single | 149,974 | 7,710.67 |
| SRR9119444 | USDA15WA-1_miseq | PE | 1,137,712 | 270.449 |

BWA-MEM (0.7.12) (9); this alignment file was sorted and indexed with SAMtools (1.4.1) (10). The PacBio assembly was polished using Pilon (1.18) and the indexed alignment file using a mindepth of 0.5 and "fix all" parameters (11). To improve the quality of the final assembly and ensure maximum correction of the remaining indel errors, an additional MiSeq paired-end (PE) shotgun library was prepared. Briefly, the strain was grown and genomic DNA extracted as described above; the sequencing libraries were prepared with the Nextera DNA Flex library preparation kit. This library was sequenced on a MiSeq platform using 2 × 300 PE reads. These additional reads were used to further polish the existing assembly using the aforementioned programs and settings. The complete genome sequence of USDA15WA-1 is 5,031,277 bp, with a GC content of 52.16%. Information for individual data files that were used to assemble the genome of USDA15WA-1 is shown in Table 1. The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

The MDR module encoding mercury tolerance and antimicrobial resistance to ampicillin, streptomycin, sulfisoxazole, and tetracycline is inserted (nucleotides 2916940 to 2945171) into the STM2759-*fljB* region, resulting in an ~15-kb deletion compared to *Salmonella enterica* serovar Typhimurium strain LT2 (NCBI RefSeq accession number NC_003197) (12). *Salmonella* genomic island 4 (SGI-4), an ~80-kb mobile genetic element containing metal tolerance genes for copper, silver, and arsenic, is located at nucleotides 4659019 to 4739704 and is flanked on both ends by a 55-bp repeat of the *pheR* tRNA (nucleotides 4658964 to 4659018 and 4739705 to 4739759, respectively) (13).

**Data availability.** The complete genome sequence of USDA15WA-1 has been deposited in GenBank under the accession number CP040686. All raw sequencing data are available from the NCBI (Table 1) under BioProject accession number PRJNA242847 and BioSample accession number SAMN04088947.

## ACKNOWLEDGMENTS

This research was partially supported by the Iowa Pork Producers Association (NPB 16-113) and USDA-ARS CRIS funds.

The mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendations or endorsement by the U.S. Department of Agriculture.

## REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.p11101.
2. Stevens MP, Humphrey TJ, Maskell DJ. 2009. Molecular insights into farm animal and zoonotic *Salmonella* infections. Philos Trans R Soc Lond B Biol Sci 364:2709–2723. https://doi.org/10.1098/rstb.2009.0094.
3. CDC. 2018. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): human isolates surveillance report for 2015 (final report). Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/narms/pdf/2015-NARMS-Annual-Report-cleared_508.pdf.
4. Kawakami VM, Bottichio L, Angelo K, Linton N, Kissler B, Basler C, Lloyd J, Inouye W, Gonzales E, Rietberg K, Melius B, Oltean H, Wise M, Sinatra J, Marsland P, Li Z, Meek R, Kay M, Duchin J, Lindquist S. 2016. Notes from the field: outbreak of multidrug-resistant *Salmonella* infections linked to pork—Washington, 2015. MMWR Morb Mortal Wkly Rep 65:379–381. https://doi.org/10.15585/mmwr.mm6514a4.
5. U.S. Department of Agriculture, Food Safety Inspection Service. 2014. FSIS sampling for the National Antimicrobial Resistance Monitoring System (NARMS). FSIS directive 10,100.1. Food Safety Inspection Service, U.S. Department of Agriculture, Washington, DC. https://www.fsis.usda.gov/wps/wcm/connect/056b7ec7-5456-4325-ae55-1a73ddd6f348/10100.1.pdf?MOD=AJPERES.
6. U.S. Department of Agriculture. 2019. Laboratory guidebook notice change of title: isolation and identification of Salmonella from meat, poultry, pasteurized egg, and Siluriformes (fish) products and carcass and environmental sponges. U.S. Department of Agriculture, Athens, GA. https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/mlg-4.pdf?MOD=AJPERES.

7. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. Genome Res 27:722–736. https://doi.org/10.1101/gr.215087.116.
8. 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.
9. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303-3997. https://arxiv.org/abs/1303.3997.
10. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
11. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
12. Garcia P, Malorny B, Rodicio MR, Stephan R, Hachler H, Guerra B, Lucarelli C. 2016. Horizontal acquisition of a multidrug-resistance module (R-type ASSuT) is responsible for the monophasic phenotype in a widespread clone of *Salmonella* serovar 4,[5],12:i. Front Microbiol 7:680. https://doi.org/10.3389/fmicb.2016.00680.
13. Petrovska L, Mather AE, AbuOun M, Branchu P, Harris SR, Connor T, Hopkins KL, Underwood A, Lettini AA, Page A, Bagnall M, Wain J, Parkhill J, Dougan G, Davies R, Kingsley RA. 2016. Microevolution of monophasic *Salmonella* Typhimurium during epidemic, United Kingdom, 2005–2010. Emerg Infect Dis 22:617–624. https://doi.org/10.3201/eid2204.150531.