



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

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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](https://doi.org/10.1128/MRA.00791-19)) 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](https://doi.org/10.1128/MRA.00791-19)) 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

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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](#).

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