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) 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
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

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REFERENCES


### Table 1

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