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GENOME SEQUENCES

Closed Genome Sequence of a *Salmonella enterica* Serotype Senftenberg Strain Carrying the *mcr-9* Gene Isolated from Broken Chicken Eggshells in Trinidad and Tobago

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ABSTRACT *Salmonella enterica* is a highly important foodborne pathogen worldwide. We report the complete genome sequence of a sequence type 14 *Salmonella enterica* serotype Senftenberg strain carrying the *mcr-9* gene in a plasmid isolated from broken chicken eggshells in Trinidad and Tobago, obtained by using a combination of long- and short-read sequencing.

Hundreds of *Salmonella* strains were isolated during a "farm-to-fork" monitoring program for the presence of antimicrobial-resistant *Salmonella* spp. in broiler chicken broken eggshells from retail outlets, processing plants, and farms in Trinidad and Tobago (2016 to 2019). Initial short-read sequencing revealed the presence of the *mcr-9* gene in one strain (UWI-H17) belonging to serovar Senftenberg. The *mcr* gene (encoding a phosphoethanolamine transferase) in *Enterobacteriaceae* has been linked to colistin resistance (1). Of the 10 *mcr* gene variants, *mcr-1* to *mcr-10* (2, 3), the *mcr-9* gene variant was initially described by Carroll et al. in 2019 (4). Here, we obtained the complete genome sequence of strain UWI-H17 using a combination of long-read and short-read sequencing technologies. Though *mcr-9* is not associated with colistin resistance in *Salmonella* and *Escherichia coli* in the United States (5), this closed genome will be useful for future outbreak investigations and studies of *mcr* gene variant dissemination in the world.

The strain was isolated and confirmed to belong to the *Salmonella* serotype Senftenberg as previously described (6, 7). For DNA extraction, the strain was grown overnight in tryptic soy broth (TSB) medium at 35°C, and the DNA was extracted using the Maxwell RSC cultured cell DNA kit (Promega, Madison, WI) following the manufacturer's protocols. The long reads for this strain were generated using a GridION sequencer (Nanopore, Oxford, UK). The sequencing library was prepared using the rapid barcoding sequencing kit (SQK-RBK004) and run in a FLO-MIN106 (R9.4.1) flow cell, according to the manufacturer's instructions, for 48 h. Default parameters were used for all software unless specified. The run was base called live with default settings (MinKNOW Core v3.6.5 and Guppy v3.2.10). The sequencing output for the sample was 646.7 Mb (179,215 reads; quality score, 9.73; N_{50}, 3,608 bp). Reads with <4,000 bp and a quality score of <7 were discarded for downstream analysis, resulting in 32,551 remaining reads for an estimated average genome coverage of 70×. The short-read whole-genome sequence for this strain was generated by MiSeq sequencing with the MiSeq v3 kit using 2 × 250-bp paired-end chemistry (Illumina, San Diego, CA), according to the manufacturer's instructions, at 70× coverage. The libraries were constructed using 100 ng of genomic DNA using the Illumina DNA prep (M) tagmentation kit, according to the manufacturer's instructions. The reads were trimmed using Trimmomatic v0.36 (8).

The initial complete genome comprising the chromosome and plasmid(s) for this strain was obtained by *de novo* assembly using Nanopore data and Flye v2.8 (9). A second hybrid assembly was generated using both Nanopore and MiSeq data with Unicycler v0.4.8

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TABLE 1 Sequence assembly statistics and other metadata for the *mcr-9*-positive *Salmonella* sequence type 14 serotype Senftenberg strain isolated from broken chicken eggshells reported in this study

CFSAN no.	Strain	Chromosome accession no.	Chromosome size (bp)	Plasmid accession no.	Plasmid size (bp)	GC content (%)	Hybrid assembly genome coverage (×)	Illumina SRA accession no.	No. of Illumina reads	Nanopore SRA accession no.	No. of Nanopore reads	AMR genes[a]
CFSAN103867	UWI-H17	CP065859	4,899,125			52.0	65	SRR11660196	1,542,894	SRR13073501	32,551	*mdsA*, *mdsB*, *mcr-9*, *aph(3″)-Ib*, *aph(6)-Id*
				CP065860	308,749	48.5	75					
				CP065861	4,096	55.5	420					
				CP065862	3,223	56.4	360					
				CP065863	3,001	48.3	430					
				CP065864	2,265	51.7	300					

[a] AMR, antimicrobial resistance. *In silico* AMR genes were found using ResFinder v4.1 (https://cge.cbs.dtu.dk/services/ResFinder/). *In silico* serotyping was conducted using SeqSero (12) (http://www.denglab.info/SeqSero), a tool to infer the serovar from the genes that determine antigenic structure. *In silico* multilocus sequence typing (MLST) used the MLST website for *Salmonella* (http://enterobase.warwick.ac.uk/species/index/senterica).

(10). The Unicycler and Flye assemblies were aligned using Mauve v2.4.0 (11). The two aligned assemblies agreed in synteny and size, and the Unicycler hybrid assembly was used as the final assembly (i.e., the complete genome). Unicycler assembled the chromosome and plasmids as circular and closed and oriented the chromosome to start at the *dnaA* gene (Table 1). The genome was annotated using the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) v5.0 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok) (13).

Data availability. The chromosome and plasmid sequences for strain UWI-H17 (CFSAN103867) are listed in Table 1.

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