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Hybrid assembled genomes of three *Salmonella enterica* isolates from municipal wastewater in Tennessee, USA

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ABSTRACT Three *Salmonella enterica* isolates were isolated from a municipal wastewater treatment plant in Knoxville, TN, USA. Genomic DNA was extracted and sequenced on the Illumina and Oxford Nanopore platforms. Annotated hybrid assemblies showed the strains were predicted to be serotypes Bareilly (n = 2) and Typhimurium (n = 1).

KEYWORDS *Salmonella*, wastewater, surveillance

Salmonella enterica is a globally widespread bacterial pathogen, causing an estimated 100 million infections and over 200,000 deaths annually (1, 2). Reporting novel *S. enterica* strains from municipal wastewater contributes genomic resources for comparative analyses and genomic surveillance. This study presents the complete and draft genome sequences of three *S. enterica* strains (UTK W1-0001, UTK W1-0003, and UTK W1-0011) isolated from municipal wastewater samples collected at a local wastewater treatment plant in Knoxville, Tennessee, USA.

Samples were collected from the primary effluent of municipal wastewater on 9 October 2023. The collected wastewater samples were filtered and enriched for *Salmonella* spp. following the modified Food and Drug Administration's Bacteriological Analytical Manual protocol (3, 4). Single colonies were isolated on selective media, and pure cultures were prepared. The presumptive *Salmonella* isolates were cultured overnight in brain heart infusion broth at 37°C with shaking at 200 rpm. DNA was extracted by the DNeasy Blood and Tissue Kit (Qiagen), assessed with Nanodrop (Thermo Fisher Scientific) and Qubit 4 (Thermo Fisher Scientific), and shipped to SeqCenter (Pittsburgh, PA, USA). Illumina short-read sequencing was performed using the Illumina DNA Prep kit and NovaSeq X Plus sequencer (Illumina), producing 151 bp paired-end reads (5). For Nanopore sequencing, DNA was extracted using the Zymo Quick-DNA HMW MagBead kit (Zymo Research Corp.). The library was prepared using the Oxford Nanopore rapid barcoding kit (SQK-RBK114.24) and sequenced with a MinION sequencer with Flongle (Oxford Nanopore Technologies). Basecalling was performed by MinKNOW (v24.06.5) using the fast basecalling model with adapter trimming, default quality score threshold, and no size-selection (6). Read quality was checked by FastQC v0.11.9 (7), and trimming was performed by Trimmomatic v0.39 (8). The hybrid assembly was performed using Unicycler v0.4.8 in bold mode, including overlap identification and trimming, with rotation to start at dnaA/repA (9). For all software, default parameters were used except where otherwise noted.

BLAST analysis (10) of the 7 kb contig in UTK W1-0011 assembly revealed 96.7% nucleotide identity across 81% of the query sequence length to plasmid NZ_CP128883.1. kSNP4 (v4.1) was used to identify core genome single-nucleotide polymorphism (SNP) among these three *Salmonella* isolates (11), revealing each was a distinct strain. Genome sequencing data and annotation features are summarized in Table 1. The genomes of strains UTK W1-0001, UTK W1-0003, and UTK W1-0011 were predicted to have 151-152 virulence factor genes. Key virulence determinants included fimbrial and

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TABLE 1 Genomic sequence statistics and features of three *S. enterica* isolates from a local wastewater treatment plant

Strain	No. of Illumina reads	No. of ONT reads	N50 of ONT reads	Total length (bp)[a]	No. of chromosome contigs[a]	No. of plasmid contigs[a]	GC content (%)[a]	Predicted serotype[b]	Illumina read coverage (×)[c]	Nanopore read coverage (×)[c]	No. of CDSs[d]	No. of tRNAs[d]	No. of CRISPR arrays[e]	Antibiotic resistance genes[f]
UTK W1-0001	2,419,110	5,000	19,045	4,964,787	1	1	52.17	Typhimurium	66	9	4,739	86	4	*aac(6')-Iaa, aph (6)-Id, blaCMY-2, sul2, tet(A), floR*
UTK W1-0003	3,066,142	8,000	17,340	4,843,513	1	1	52.05	Bareilly	90	14	4,648	85	3	*aac(6')-Iaa*
UTK W1-0011	2,943,720	7,000	17,533	5,032,162	1	2	52.14	Bareilly	83	12	4,871	86	2	*aac(6')-Iaa*

[a]Assembly statistics and quality were generated using QUAST v5.2.0 (16) and CheckM v1.1.3 (17).
[b]The species and serotype were analyzed by EnteroBase v1.2.0 (18).
[c]Average read coverage was determined using Minimap2 v2.14 (19), BBMap v38.63 (20), and SAMtools v1.15.1 (21).
[d]The assemblies were annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP, v6.8) (22).
[e]The clustered regularly interspaced short palindromic repeat (CRISPR) arrays were predicted using CRISPRFinder (23, 24).
[f]Antimicrobial resistance and virulence genes were screened using ResFinder v4.6.0 (25) and Virulence Factor Database (VFDB) (26).

nonfimbrial adherence determinants, macrophage inducible gene mig-14, and type III secretion system (T3SS) encoded by *Salmonella* pathogenicity island 1 (SPI-1) and 2 (SPI-2). Compared with previously isolated *Salmonella enterica* strains harboring 100-130 virulence genes identified by VFDB (12–15), the three newly isolated *Salmonella enterica* strains exhibit a notable virulence profile potentially indicative of aggressive pathogenic characteristics.

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DATA AVAILABILITY

The genomic sequencing data and assemblies for strains UTK W1-0001, UTK W1-0003 and UTK W1-0011 have been deposited in the NCBI database under BioProject number PRJNA1169800. The corresponding BioSample numbers are SAMN44082895, SAMN44082896, and SAMN44082897; SRA accession numbers are SRR30900535, SRR30900538, SRR30900534, SRR30900537, SRR30900533, and SRR30900536. Assembly accession numbers are CP171353.1, CP171354.1, CP171351.1, CP171352.1, and JBJDRN000000000.1.

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