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GENOME SEQUENCES

# Closed Genome Sequences and Antimicrobial Resistance Profiles of Eight Wild Bird *Salmonella* Isolates Obtained with MinION and Illumina MiSeq Sequencing

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**ABSTRACT** Complete genome sequences of eight isolates of *Salmonella enterica* subsp. *enterica* from Canadian wild birds were determined by MinION and Illumina MiSeq sequencing. Assembled chromosomes had an average size of 4,833,662 bp. *Salmonella enterica* serovar Worthington obtained from partridge and quail carried 267-kb plasmids, which contained multiple antimicrobial resistance genes.

Multidrug antimicrobial resistance (AMR) in *Salmonella* species is considered a global public health threat (1–3), and there is a need to develop microbial sequence resources to evaluate possible contributions by *Salmonella* strains of wild bird origin. We sequenced eight *Salmonella* organisms isolated from Canadian wild birds from the provinces of British Columbia, Ontario, Saskatchewan, and Newfoundland and Labrador. The organisms were isolated using a combination of primary enrichment culture in peptone broth at 37°C overnight, followed by inoculation in Rappaport-Vassiliadis selective enrichment broth at 42°C overnight, and plating onto XLT-4 selective agar to grow the bacterial colonies (Table 1). High-quality DNA was extracted from an overnight culture (1 ml of brain heart infusion medium at 37°C) using the Wizard genomic DNA purification kit (Promega, Madison, WI) and assessed with a spectrophotometer (DU 730; Beckman Coulter, Mississauga, ON, Canada) and a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA). Library construction for Illumina MiSeq sequencing was carried out with the Nextera XT DNA kit (Illumina, San Diego, CA), and sequencing and read trimming were performed as described (4). Libraries for MinION sequencing were prepared without shearing using the 1D ligation sequencing kit (SQK-LSK108), and DNA was barcoded with the native barcoding expansion kit (EXP-NBD103) according to the manufacturer's instructions (Oxford Nanopore Technologies, Inc., Oxford, UK). The final library was analyzed by MinION sequencing on a FLO-MIN106 (R9.4.1) flow cell for 48 h. Fast5 reads were basecalled using the high-accuracy basecalling algorithm in Guppy (v3.1.5), and the resulting fastq reads were trimmed with Porechop v0.2.3 (default settings) and filtered with Filtlong v0.2.0, keeping the top 90% quality reads or reads with 100× coverage. Hybrid assembly of Illumina paired-end reads and MinION reads was achieved with SPAdes v3.11.1 (5) and polished with Pilon v1.23 (6) using Unicycler v0.4.4 (7). Overlapping regions were trimmed and the genomes were rotated using the fixstart program in Circlator (8). The genomes were quality checked with QUAST v5.0.2 (9), and the depth of sequencing coverage was determined by mapping individual reads against the assembled genomes using minimap2 v2-2.17, with visualization using Qualimap v2.2.1. The presence of AMR genes was determined with ResFinder v3.0 (10). Default parameters were used for all analyses except where otherwise noted. Assembled genomes and raw reads were submitted to GenBank (Table 1). Each polished genome contained a single chromosome and frequently multiple contigs representing plasmid and/or phage sequences. Six isolates (ST-13, ST-29, ST-32, ST-33, ST-35, and ST-87) were identified as *Salmonella*

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**TABLE 1** Source information and genomic characteristics of *Salmonella enterica* subsp. *enterica* isolates from Canadian wild birds

| Strain | Sample name | Source | Province[a] | Serovar | Median read length (bp) for: | | No. of contigs | Chromosome size (bp) | Plasmid size(s) (bp) | Bacteriophage size (bp) | GC content (assembled molecule) (%) | Depth of coverage (×) | GenBank accession no. for whole molecule(s) | SRA accession no. for MiSeq/MinION reads |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | MiSeq reads | MinION reads | | | | | | | | |
| ST-13 | OLF_FSR1_WB_Finch_ST-13 | Finch | BC | Typhimurium | 300 | 9,002 | 1 | 4,819,669 | —[b] | — | 52.15 | 99 | CP051269 | SRX7862684/ SRX7862698 |
| ST-29 | OLF-FSR1_WB_Gull_ST-29 | Gull | SK | Typhimurium | 295 | 3,916 | 3 | 4,879,541 | 94,038 | 41,384 | 52.19 | 59 | CP051286, CP051287, CP051288 | SRX7862685/ SRX7862699 |
| ST-32 | OLF-FSR1_WB_Gull_ST-32 | Gull | ON | Typhimurium | 300 | 2,823 | 2 | 4,920,253 | — | 41,336 | 52.14 | 52 | CP051284, CP051285 | SRX7862692/ SRX7862686 |
| ST-33 | OLF-FSR1_WB_Hawk_ST-33 | Hawk | ON | Typhimurium | 301 | 5,468 | 2 | 4,818,617 | 93,798 | — | 52.18 | 99 | CP051267, CP051268 | SRX7862693/ SRX7862687 |
| ST-35 | OLF-FSR1_WB_Junco_ST-35 | Junco | BC | Typhimurium | 300 | 4,312 | 5 | 4,820,281 | 99,574, 95,663, 6,035 | 45,661 | 52.13 | 98 | CP051279, CP051280, CP051281, CP051282, CP051283 | SRX7862694/ SRX7862688 |
| ST-87 | OLF-FSR1_WB_Sparrow_ST-87 | Sparrow | NL | Typhimurium | 301 | 5,311 | 3 | 4,697,589 | 92,921 | 41,335 | 51.95 | 65 | CP051276, CP051277, CP051278 | SRX7862695/ SRX7862689 |
| SW-37 | OLF-FSR1_WB_Partridge_SW-37 | Partridge | BC | Worthington | 283 | 4,336 | 3 | 4,759,747 | 267,109 | 41,874 | 51.86 | 95 | CP051273, CP051274, CP051275 | SRX7862696/ SRX7862690 |
| SW-70 | OLF-FSR1_WB_Quail_SW-70 | Quail | BC | Worthington | 300 | 3,440 | 3 | 4,857,600 | 267,106 | 42,910 | 52.14 | 98 | CP051270, CP051271, CP051272 | SRX7862697/ SRX7862691 |

[a] BC, British Columbia; SK, Saskatchewan; ON, Ontario; NL, Newfoundland and Labrador.
[b] —, absent.

*enterica* subsp. *enterica* serovar Typhimurium, while the remaining two isolates (SW-37 and SW-70, obtained from a partridge and a quail, respectively) were identified as *Salmonella enterica* subsp. *enterica* serovar Worthington. The average chromosome size was 4,833,662 bp. The large virulence plasmid of *S.* Typhimurium was found in isolates ST-29, ST-33, ST-35, and ST-87. Apart from the large plasmid, isolate ST-35 contained an additional 6,050-bp *Salmonella*-specific plasmid and a 95,814-bp sequence, identified as plasmid pEC006 from *Escherichia coli* by BLAST searching of the nucleotide database. Both *S.* Worthington isolates (SW-37 and SW-70) contained a very large 267-kb plasmid with AMR genes for aminoglycosides [*aph(3′)-Ia* and *aadA7*], tetracycline [*tet*(B)], and sulfonamides (*sul1*).

**Data availability.** Raw reads and assembled genomes were submitted to GenBank under BioProject number PRJNA605433 and the accession numbers are provided in Table 1.

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