GENOME SEQUENCES

Complete Closed Genome Sequences of Three *Salmonella enterica* subsp. *enterica* Serovar Dublin Strains Isolated from Cattle at Harvest

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ABSTRACT *Salmonella enterica* subsp. *enterica* serovar Dublin is a host-adapted pathogen for cattle that can cause invasive disease in humans. To facilitate genomic comparisons characterizing virulence determinants of this pathogen, we present the complete genome sequences of three *S.* Dublin strains isolated from bovine sources at harvest.

Salmonella enterica infection is a leading cause of enterocolitis for humans and animals (1). While *Salmonella* infections are usually self-limiting, invasive infection can occur in susceptible populations (infants, elderly, and immunocompromised individuals), and when the infection is caused by drug-resistant *Salmonella* spp., treatment options are limited. *Salmonella enterica* subsp. *enterica* serovar Dublin is noted as a cattle-adapted *Salmonella* serotype, but it can cause disease in humans. A recent report on the epidemiology of *S.* Dublin demonstrated an increasing trend toward multidrug resistance (MDR) in strains isolated from humans in the United States from 1968 to 2013 and that infections caused by this serotype are more likely to be invasive than infections caused by other common *Salmonella* serotypes (2). However, the genetic determinants contributing to this invasive phenotype are not well understood. To facilitate comparative genomic studies examining the virulence traits and drug resistance determinants of this serotype, we present the complete closed genome and plasmid sequences for three MDR *S.* Dublin strains, with different plasmid content and/or drug resistance profiles. Strains 69807 and 69840 contain virulence plasmids (pSD2-69807 and pSD1-69840, respectively) that are nearly identical (99.96% pairwise identity) to the virulence plasmid in *S.* Dublin OU7409 and pSDVr (GenBank accession number DQ115388) (3), as well as IncA/C2 resistance plasmids (pSD1-69807 and pSD2-69840, respectively) harboring the resistance genes noted in Table 1. All strains were isolated from healthy cattle at harvest and confirmed as *S.* Dublin with antibody agglutination (4–6). Strains were cultured on tryptic soy agar at 37°C, and their susceptibility to 15 antimicrobial agents (as defined in the footnote of Table 1) was determined using the CMV2AGNF National Antimicrobial Resistance Monitoring System (NARMS) test panel (Sensititre, Trek Diagnostics, Thermo Fischer), following manufacturer and Clinical and Laboratory Standards Institute guidelines (7). Genomic DNA was purified with the Qiagen Genomic-tip 100/G columns and blood and cell culture DNA midi kits (Qiagen, Valencia, CA), using the manufacturer's recommended protocol for overnight cultures grown statically at 37°C in tryptic soy broth (Becton, Dickinson, Franklin Lakes, NJ). Single-molecule real-time sequencing libraries of bacterial DNA were constructed per the manufacturer's protocol using C4/P6 (chemistry/polymerase) and sequenced using a Pacific Bioscience (PB) RS II instrument (Menlo Park, CA), producing average subreads of >5 kb and mean genome coverage of 159×. Genomes

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TABLE 1 Chromosome and plasmid sequence accession numbers and additional information for 3 *Salmonella enterica* subsp. *enterica* serovar Dublin strains isolated from cattle at harvest[a]

Strain or plasmid	MLST	GenBank accession no. (SRA accession no.)	No. of reads (fold coverage)	Size (bp)	% GC content	AMR phenotype	Inc type	Resistance gene(s)[b]	Isolation yr
USMARC-69807[c]	ST10	CP032379 (SRR8063632)	75,195 (150×)	4,844,213	52.2	AmApFT (Ax)CSSuTe		*aac(6′)-Iaa*[d]	2006[e]
pSD1-69807		CP032380	1,058 (74×)	96,567	52.8		IncA/C2	bla_{CMY-2}, *sul2*, bla_{TEM-81}, *floR*, *aph(3′′)-Ib*, *aph(6)-Id*, *tet*(C)	
pSD2-69807		CP032381	893 (66×)	74,562	48.6		IncX/IncFII	*vga*(C)	
USMARC-69838[c,f]	ST10	CP032449 (SRR8064828)	71,397 (150×)	4,913,018	52.2	AmApFTAxKCSSuTe		bla_{CMY-2}, bla_{CMY-2}, *sul2*, bla_{TEM-81}, *aph(6)-Id*, *aph(3′′)-Ib*, *aph(3′)-Ia*, *aac(6′)-Iaa*[d], *floR*, *tet*(C)	2012[g]
pSD1-69838		CP032450	3,196 (195×)	114,923	49.5		IncA/C2; IncX/IncFII	*vga*(C)	
USMARC-69840[c]	ST10	CP032446 (SRR8034318)	83,212 (176×)	4,844,133	52.2	CKNaSSuTe		*aac(6′)-Iaa*[d]	2012[g]
pSD1-69840		CP032447	1,275 (106×)	74,560	48.6		IncX; IncFII(s)	*vga*(C)	
pSD2-69840		CP032448	652 (70×)	77,171	53.4		IncA/C2	*floR*, *aph(6)-Id*, *aph(3′′)-Ib*, *aph(3′)-Ia*, *sul2*, *tet*(C)	

[a] ST, sequence type; AMR, antimicrobial resistance; Am, amoxicillin-clavulanic acid; Ap, ampicillin; F, cefoxitin; Ax, ceftriaxone; C, chloramphenicol; K, kanamycin; S, streptomycin; Su, sulfisoxazole; Sxt, sulfamethoxazole-trimethoprim; T, ceftiofur; Te, tetracycline; (), indicates intermediate resistance.
[b] Resistance genes were identified using the Comprehensive Antibiotic Resistance Database (version 2.0.3) Resistance Gene Identifier (version 4.2.0) (13).
[c] *Salmonella enterica* subsp. *enterica* serovar Dublin strain.
[d] May be phenotypically silent (cryptic) and not generally noted as conferring aminoglycoside resistance (14).
[e] Source: pre-evisceration carcass.
[f] Two copies of bla_{CMY-2} present, DZA56_07580 and DZA56_07860.
[g] Source: subiliac lymph node.

were assembled using the hierarchical genome assembly protocol (HGAP) version 3.0 with a minimum seed length of 6,000 (8). For each contig, a dot plot was constructed using Geneious version 11.1.3 (Biomatters Ltd., New Zealand) (9) to identify the overlapping region, which was trimmed from the 3′ end of the contig. OriFinder (10) was used to identify the origin of replication, which was set as nucleotide position 1. The trimmed and newly oriented sequences were validated using the PB RS_Resequencing pipeline to map the corresponding reads back to the new reference in order to generate consensus concordance assemblies (8). Plasmid Inc types were determined using the *in silico* typing tool PlasmidFinder version 1.3 (default settings, 95% identity [ID] threshold and 60% minimum length) (11). Multilocus sequence types (MLST) and MDR genotypes were determined using *Salmonella in silico* typing resource (SISTR) (12) and the Comprehensive Antibiotic Resistance Database (CARD) (13), respectively. Genome and plasmid sequence data, as well as methylation data, were deposited into NCBI GenBank. Sequence data were annotated using the NCBI Prokaryotic Genome Annotation Pipeline.

Data availability. Accession numbers (for assemblies and raw reads), MLST, sizes, source data, plasmid Inc types, GC contents, and phenotypic and genotypic antimicrobial resistance phenotypes of the strains are listed in Table 1.

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