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Complete Genome Sequences of 14 *Salmonella enterica* Serovar Enteritidis Strains Recovered from Human Clinical Cases between 1949 and 1995 in the United States

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ABSTRACT *Salmonella enterica* serovar Enteritidis is one of the most commonly isolated foodborne pathogens and is transmitted primarily to humans through consumption of contaminated poultry and poultry products. We are reporting completely closed genome and plasmid sequences of historical *S.* Enteritidis isolates recovered from humans between 1949 and 1995 in the United States.

An estimated 1.2 million people in the United States contract salmonellosis annually, resulting in 23,128 hospitalizations and >452 deaths (1). Since the 1980s, *Salmonella enterica* serovar Enteritidis has been one of the most common *Salmonella* serovars causing foodborne salmonellosis in the United States. *S.* Enteritidis is often transmitted to humans through contaminated eggs and other poultry products (2–6).

The lack of genetic variation within the serovar limits the use of conventional molecular subtyping methods for outbreak investigation and source attribution. Whole-genome sequencing is a viable platform for distinguishing outbreak isolates from epidemiologically distinct, but genetically related, *S.* Enteritidis strains (7). The availability of fully characterized and sequenced historical *S.* Enteritidis reference sequences significantly helps to increase our understanding of the evolution of this serovar. To facilitate this, we sequenced 14 *S.* Enteritidis isolates recovered from human clinical cases between 1949 and 1995 and released completely closed chromosomes and plasmids.

S. Enteritidis isolates were cultured in Trypticase soy broth (Becton Dickinson, Franklin Lakes, NJ, USA) at 37°C overnight. The genomic DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Inc., Valencia, CA, USA) per the manufacturer's protocol. The genomes were sequenced using the PacBio RS II sequencing platform, as previously reported (8). The library was prepared based on the 20-kb PacBio sample preparation protocol and sequenced using P6/C4 chemistry on four single-molecule real-time (SMRT) cells with a 240-min collection time. The continuous long-read (CLR) data were *de novo* assembled using the PacBio hierarchical genome assembly process (HGAP) (version 3.0) with default parameters (4). The assembled sequences were annotated using the NCBI Prokaryotic Genomes Annotation Pipeline v4 (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) and deposited at GenBank.

The *S.* Enteritidis genomes ranged between 4,661,885 and 4,934,512 bases and were fully closed, with coverage ranging between 119× and 517×. Multiple plasmids with sizes ranging between 33 kb and 244 kb were identified in some of the isolates. All plasmid sequences identified were completely closed. Resistome analysis was conducted using the publicly available resistance gene database, ResFinder, using a 90% sequence identity and 60% length minimum to identify acquired resistance genes (9). A summary of coverages, annotations, phenotypes, and resistomes is shown in Table 1.

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TABLE 1 Summary of genome sequence analysis and antimicrobial resistance genes identified in *S. enteritidis*

S. enteritidis strain	Yr of collection	GenBank accession no.	Chromosome/plasmid	Genome/ plasmid size (bp)	Coverage (×)	Total no. of genes	Total no. of CDS[a]	No. of CDS excluding pseudogenes	No. of RNA genes	Resistance phenotype[b]	Antimicrobial resistance genes identified
49-2444	1949	CP018633	Chromosome	4,661,885	149	4,846	4,726	4,519	120	Pan-susceptible	
		CP018634	Plasmid pSE49-2444	92,891	101						
56-3991	1956	CP018635	Chromosome	4,678,115	410	4,838	4,715	4,576	123	STR	
		CP018636	Plasmid pSE56-3991	59,373	34						
69-3861	1969	CP018637	Chromosome	4,690,881	423	4,994	4,874	4,709	120	AMP, FIS	bla_{OXA2}, *sul1*
		CP018638	Plasmid pSE69-3861-1	95,773	126						
		CP018639	Plasmid pSE69-3861-2	59,335	45						
70-1605	1970	CP018640	Chromosome	4,679,538	478	4,832	4,709	4,570	123	STR	
		CP018641	Plasmid pSE70-1605	59,371	48						
74-1357	1974	CP018642	Chromosome	4,698,044	377	4,927	4,805	4,637	122	AMP, STR, TET	*aph*(3′)-*Ib*, *aph*(6)-*Id*, bla_{TEM-1}, *tetA*
		CP018643	Plasmid pSE74-1357	118,824	1,409						
77-2980	1977	CP018644	Chromosome	4,686,092	434	4,755	4,635	4,400	120	AMP, STR, TET	
79-2359	1979	CP018645	Chromosome	4,684,908	270	4,827	4,703	4,499	124	AMP, STR	*aph*(6)-*Id*, *aph*(3′)-*Ib*, bla_{TEM-1}
		CP018646	Plasmid pSE79-2359	54,796	50						
81-1435	1981	CP018647	Chromosome	4,699,764	119	4,789	4,667	4,515	122	Pan-susceptible	
81-1607	1981	CP018648	Chromosome	4,688,972	517	4,898	4,776	4,626	122	AMP, STR, TET, FIS	*aph*(6)-*Id*, *aph*(3′)-*Ib*, bla_{TEM-1}
		CP018649	Plasmid pSE81-1607-1	54,541	202						
		CP018650	Plasmid pSE81-1607-2	59,372	114						
81-1705	1981	CP018651	Chromosome	4,698,445	424	5,152	5,022	4,835	130	AMP, CHL, STR, TET, FIS	*aph*(6)-*Id*, *aph*(3′)-*Ib*, bla_{TEM-1}, *cat1*, *tetD*, *tetC*, *tetB*, *sul2*
		CP018652	Plasmid pSE81-1705-1	200,045	276						
		CP018653	Plasmid pSE81-1705-2	59,334	36						
		CP018654	Plasmid pSE81-1705-3	33,785	430						
81-1706	1981	CP018655	Chromosome	4,699,800	307	5,091	4,969	4,753	122	AMP, CHL, STR, TET	*aph*(6)-*Id*, *aph*(3′)-*Ib*, bla_{TEM-1}, *cat1*, *tetB*, *tetC*, *tetD*
		CP018656	Plasmid pSE81-1706	244,210	415						
92-0392	1992	CP018657	Chromosome	4,934,512	286	5,202	5,082	4,900	120	AMP, STR, TET, FIS	*aadA2*, bla_{carB-2}, *sul1*, *tetG*, *floR*
		CP018658	Plasmid pSE92-0392	94,039	86						
93-0639	1993	CP018659	Chromosome	4,679,307	304	4,838	4,717	4,528	124	Pan-susceptible	
		CP018660	Plasmid pSE93-0639	59,370	66						
95-0621	1995	CP018661	Chromosome	4,679,624	221	4,893	4,771	4,593	122	AMP, TET	bla_{TEM-1}, *tetA*
		CP018662	Plasmid pSE95-0621-1	59,356	28						
		CP018663	Plasmid pSE95-0621-2	51,980	48						

[a]CDS, coding sequences.

[b]AMP, ampicillin; CHL, chloramphenicol; STR, streptomycin; FIS, sulfisoxazole; TET, tetracycline.

Accession number(s). The *S.* Enteritidis and plasmid sequences reported in this study have been deposited in the DDBJ/ENA/GenBank database under the accession numbers indicated in Table 1.

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REFERENCES

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis 17:7–15. https://wwwnc.cdc.gov/eid/article/17/1/P1-1101_article.
2. Jackson BR, Griffin PM, Cole D, Walsh KA, Chai SJ. 2013. Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. Emerg Infect Dis 19:1239–1244. https://doi.org/10.3201/eid1908.121511.
3. Rodrigue DC, Tauxe RV, Rowe B. 1990. International increase in *Salmonella enteritidis*: a new pandemic? Epidemiol Infect 105:21–27. https://doi.org/10.1017/S0950268800047609.
4. Chai SJ, White PL, Lathrop SL, Solghan SM, Medus C, McGlinchey BM, Tobin-D'Angelo M, Marcus R, Mahon BE. 2012. *Salmonella enterica* serotype Enteritidis: increasing incidence of domestically acquired infections. Clin Infect Dis 54(Suppl 5):S488–S497. https://doi.org/10.1093/cid/cis231.
5. Braden CR. 2006. *Salmonella enterica* serotype Enteritidis and eggs: a national epidemic in the United States. Clin Infect Dis 43:512–517. https://doi.org/10.1086/505973.
6. Trepka MJ, Archer JR, Altekruse SF, Proctor ME, Davis JP. 1999. An increase in sporadic and outbreak-associated *Salmonella* enteritidis infections in Wisconsin: the role of eggs. J Infect Dis 180:1214–1219. https://doi.org/10.1086/314984.
7. den Bakker HC, Allard MW, Bopp D, Brown EW, Fontana J, Iqbal Z, Kinney A, Limberger R, Musser KA, Shudt M, Strain E, Wiedmann M, Wolfgang WJ. 2014. Rapid whole-genome sequencing for surveillance of *Salmonella enterica* serovar enteritidis. Emerg Infect Dis 20:1306–1314. https://doi.org/10.3201/eid2008.131399.
8. Yao K, Muruvanda T, Roberts RJ, Payne J, Allard MW, Hoffmann M. 2016. Complete genome and methylome sequences of two *Salmonella enterica* spp. Genome Announc 4(1):e01599-15. https://doi.org/10.1128/genomeA.01599-15.
9. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.