



Whole-Genome Sequences of 66 Incompatibility Group FIB Plasmid-Carrying *Salmonella enterica* Serovar Typhimurium Isolates from Food Animal Sources

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ABSTRACT Sixty-six *Salmonella enterica* serovar Typhimurium isolates carrying incompatibility group FIB (IncFIB) plasmids were sequenced to further characterize the IncFIB plasmid-encoded factors associated with virulence and antimicrobial resistance genes. In addition to the IncFIB plasmid, many of these isolates harbored additional plasmids encoding virulence and antimicrobial resistance genes.

Salmonella enterica, a major foodborne pathogen, has been identified as the source of multiple outbreaks linked to contaminated foods or pet animals in the United States (1, 2). Among 2,600 *Salmonella* serovars identified, Typhimurium is one of the predominant serovars that can cause illness in animals and humans (3, 4). *S. enterica* contains arrays of virulence factors commonly encoded on *Salmonella* pathogenicity islands (SPIs) located on the chromosome (3, 4). Additionally, some plasmids, including incompatibility group FIB (IncFIB) plasmids, are known to encode virulence factors and antimicrobial resistance phenotypes (5, 6). However, much remains to be studied regarding plasmid factors that give bacteria enhanced capacity to compete in food animal environments and cause human infections. The aim of this study was to evaluate the antimicrobial resistance and virulence gene content of a historical collection of IncFIB-positive *S. enterica* serovar Typhimurium isolates. Sixty-six *S. enterica* serovar Typhimurium isolates originating from chickens ($n = 32$, 48%), turkeys ($n = 18$, 27%), cattle ($n = 10$, 15%), swine ($n = 4$, 6%), and poultry water ($n = 2$, 3%) were sequenced. These isolates were collected from different locations in the United States in 1999 and 2003.

S. Typhimurium isolates were identified and phenotypically characterized using standard microbiological methods (4). Sixty-six *S. Typhimurium* isolates identified as carrying the IncFIB plasmid using PCR-based plasmid replicon typing were sequenced (7). Each isolate was stored at -80°C in brain heart infusion broth (Remel, Lenexa, KS) containing 20% glycerol and subsequently subcultured on blood agar plates (tryptic soy agar with 5% sheep's blood [Remel]) and incubated at 35°C for 24 hours. After overnight incubation, bacterial growth was collected from the plate using a $1\text{-}\mu\text{l}$ inoculating loop and added to $180\ \mu\text{l}$ animal tissue lysis (ATL) buffer (Qiagen, Valencia, CA, USA) (8). The bacterial genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen). A Nanodrop instrument and a Qubit broad-range (BR) assay kit were used to measure the quality and quantity of the DNA (Thermo Fisher Scientific, Grand Island, NY, USA). DNA sequencing libraries were constructed with the Nextera XT DNA library preparation kit (Illumina, San Diego, CA), and samples were multiplexed with a combination of two indexes of the Nextera XT index kit. Whole-genome sequencing (WGS) reactions were performed on an Illumina MiSeq instrument with a $2 \times 250\text{-bp}$ paired-end format (8).

The raw sequence data were trimmed, and *de novo* assembly was completed using

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TABLE 1 Whole-genome sequencing analyses of 66 *S. Typhimurium* isolates

Isolate	Source	Yr of isolation	No. of sequence reads	N_{50} (bp)	No. of contigs	Genome length (bp)	No. of CDSs	G+C content (%)	GenBank accession no.
368	Cattle	1999	5,002,066	213,643	398	5,066,745	5,443	52.15	PDN000000000
373	Cattle	1999	5,004,420	217,199	238	5,030,898	5,260	52.16	PDNN000000000
374	Cattle	1999	5,266,652	41,074	313	5,266,238	5,494	52.15	VTSM000000000
375	Cattle	1999	5,245,598	25,902	463	5,244,985	5,512	52.21	VTSL000000000
376	Chicken	1999	4,908,443	191,467	194	4,937,820	5,071	52.14	PDNM000000000
391	Chicken	1999	5,272,802	22,706	512	5,272,682	5,509	51.78	VTSK000000000
393	Swine	1999	4,942,202	75,759	212	4,942,202	5,036	52.17	VSXW000000000
411	Chicken	1999	5,050,348	26,129	439	5,050,348	5,195	52.07	VSXV000000000
417	Chicken	1999	5,048,011	32,748	318	5,048,011	5,200	51.99	VSXU000000000
419	Swine	1999	4,866,988	24,572	460	4,866,988	5,000	52.19	VSXT000000000
420	Chicken	1999	5,089,876	38,056	300	5,089,876	5,218	51.95	VSXS000000000
421	Chicken	1999	5,159,285	36,172	367	5,159,285	5,377	51.92	VSXI000000000
423	Chicken	1999	5,123,919	38,233	286	5,123,919	5,296	51.97	VSXR000000000
426	Turkey	1999	5,367,202	94,863	207	4,937,820	5,071	52.14	PDNK000000000
427	Chicken	1999	4,983,376	49,847	241	4,983,376	5,093	52.03	VSXQ000000000
443	Turkey	1999	5,035,390	34,401	334	5,035,390	5,178	52.15	VSXP000000000
447	Turkey	1999	5,034,323	35,622	338	5,034,323	5,172	52.12	VSXO000000000
448	Chicken	1999	5,150,521	31,437	394	5,150,521	5,320	51.74	VSXN000000000
449	Chicken	1999	4,935,787	25,136	426	4,935,787	5,071	52.08	VSXM000000000
452	Turkey	1999	5,037,174	127,526	524	5,134,028	5,495	52.18	LYRS000000000
455	Chicken	1999	4,985,161	36,795	288	4,985,161	5,117	52.17	VSXL000000000
457	Turkey	1999	5,016,689	26,345	429	5,016,689	5,142	52.15	VSXH000000000
458	Turkey	1999	4,945,793	27,769	373	4,945,793	5,047	52.15	VSXK000000000
459	Chicken	1999	5,123,374	34,100	345	5,122,989	5,276	52.02	VTSH000000000
460	Turkey	1999	5,057,342	26,483	435	5,056,931	5,200	52.12	VTSI000000000
462	Turkey	1999	5,031,597	162,420	108	5,027,837	5,137	52.11	VTSU000000000
463	Swine	1999	5,029,041	104,076	638	5,150,184	5,556	51.82	PHGW000000000
464	Turkey	1999	4,994,827	24,882	435	4,994,827	5,146	52.19	VSXG000000000
465	Turkey	1999	5,039,004	36,760	375	5,038,704	5,195	52.11	VTSG000000000
475	Swine	1999	4,699,749	46,241	214	4,699,749	4,765	52.14	VSXJ000000000
477	Turkey	1999	5,019,363	29,981	380	5,019,152	5,171	52.12	VTSF000000000
481	Turkey	1999	4,980,621	31,822	349	4,980,621	5,099	52.13	VSXF000000000
483	Turkey	1999	5,015,525	35,497	346	5,015,405	5,134	52.11	VTSD000000000
484	Turkey	1999	5,005,022	61,349	249	5,037,453	5,198	52.09	PDOI000000000
485	Turkey	1999	5,012,760	47,950	242	5,012,760	5,119	52.11	VSXE000000000
486	Turkey	1999	5,003,423	32,782	335	5,003,124	5,137	52.17	VTSC000000000
487	Turkey	1999	5,009,634	30,447	400	5,009,634	5,178	52.22	VSXD000000000
489	Turkey	1999	5,012,284	36,322	312	5,012,164	5,160	52.19	VTSB000000000
492	Cattle	1999	5,096,032	225,464	190	5,114,958	5,344	52.05	PDOJ000000000
494	Cattle	1999	5,177,448	225,613	163	5,193,385	5,432	52.16	PDOK000000000
495	Cattle	1999	5,015,521	36,243	290	5,015,461	5,160	52.18	VTSA000000000
498	Cattle	1999	5,332,897	38,106	365	5,332,897	5,663	52.02	VSXC000000000
499	Cattle	1999	5,048,722	53,492	234	5,048,588	5,206	52.03	VTRZ000000000
500	Cattle	1999	5,008,507	29,084	354	5,008,507	5,152	52.18	VSXB000000000
N028	Chicken	2003	5,336,122	39,125	323	5,336,122	5,564	51.67	VSWY000000000
N029	Chicken	2003	5,315,626	27,154	466	5,315,626	5,567	51.72	VSXA000000000
N030	Chicken	2003	5,190,355	37,910	325	5,190,355	5,399	51.90	VSWX000000000
N032	Chicken	2003	5,323,303	36,540	295	5,323,303	5,585	51.68	VSWZ000000000
N033	Chicken	2003	5,336,039	33,241	335	5,335,859	5,570	51.67	VTRY000000000
N034	Chicken	2003	5,193,936	39,096	302	5,193,936	5,393	51.89	VSWW000000000
N035	Chicken	2003	5,320,318	40,627	297	5,320,318	5,547	51.68	VSWV000000000
N061	Chicken	2003	5,090,575	57,542	219	5,090,575	5,257	51.91	VSWU000000000
N062	Chicken	2003	5,078,622	35,133	311	5,078,388	5,226	51.93	VTRX000000000
N063	Chicken	2003	5,091,393	60,958	203	5,091,393	5,242	51.90	VSWT000000000
N065	Chicken	2003	5,092,901	55,292	226	5,092,901	5,248	51.90	VSWS000000000
N066	Chicken	2003	5,089,662	46,401	272	5,089,662	5,250	51.91	VSWR000000000
N067	Chicken	2003	5,096,841	49,921	228	5,096,841	5,263	51.89	VSWQ000000000
N068	Chicken	2003	5,093,904	52,142	256	5,093,904	5,282	51.91	VSWP000000000
N069	Chicken	2003	5,174,143	55,555	230	5,174,143	5,349	52.03	VSWO000000000
N070	Chicken	2003	5,103,058	63,801	222	5,103,058	5,277	51.87	VSWN000000000
N071	Chicken	2003	5,089,277	48,505	244	5,089,277	5,258	51.91	VSWM000000000
N073	Chicken	2003	5,117,766	57,646	266	5,117,570	5,297	51.85	VTRW000000000
N075	Chicken	2003	5,086,937	65,664	209	5,086,937	5,248	51.91	VSWL000000000
N135	Poultry water	2003	5,080,118	60,945	187	5,080,118	5,220	51.96	VSWK000000000
N138	Poultry water	2003	5,072,327	31,198	363	5,072,327	5,236	51.99	VSWJ000000000
N140	Chicken	2003	5,181,139	43,433	267	5,181,139	5,364	51.99	VSWI000000000

CLC Genomics Workbench version 9.0 (Qiagen, Redwood City, CA). Sequences were annotated initially using the Pathosystems Resource Integration Center (PATRIC) software version 3.5.36 (9) and then submitted to NCBI for final annotation with the Prokaryotic Genome Annotation Pipeline (PGAP) (10). The number of contigs, genome length (bp), coding sequences (CDSs), and G+C content of each sample were annotated via PATRIC as listed in Table 1. The final annotations performed using PGAP are available at NCBI under the accession numbers shown in Table 1. The WGS results were further analyzed using PlasmidFinder (11), ResFinder (12), and the PATRIC_VF database (9) to characterize and identify the antimicrobial resistance and putative virulence genes carried by the IncFIB plasmids. Each of the analyses was performed using default parameter settings.

PlasmidFinder analyses showed that all of the isolates contained IncFIB plasmids along with other plasmid replicon types, including IncColpVC ($n = 43$, 65%), IncFIC(FII) ($n = 34$, 52%), IncI1 ($n = 30$, 45%), IncFIA ($n = 19$, 29%), IncA/C2 ($n = 16$, 24%), IncFII(pcoo) ($n = 15$, 23%), IncFII(S) ($n = 14$, 21%), IncX1 ($n = 11$, 17%), IncHI2A ($n = 8$, 12%), and IncHI2 ($n = 7$, 11%). Multiple antimicrobial resistance genes, including *bla*_{CMY-2} in 65% ($n = 43$) of the total isolates, followed by *tet*(A) ($n = 27$, 40%), *tet*(B) ($n = 24$, 36%), *bla*_{TEM-1B} ($n = 22$, 33%), *sul2* ($n = 19$, 29%), *sul1* ($n = 17$, 26%), *tet*(G) ($n = 2$, 3%), and *bla*_{TEM} ($n = 1$, 1.5%), were identified. WGS analysis using PATRIC identified several virulence genes, including iron acquisition genes, such as *iroN* ($n = 66$, 100%), *iutA* ($n = 65$, 98%), *sitA* ($n = 65$, 98%), and *iucA* ($n = 49$, 74%). Additionally, all isolates contained chromosomally encoded *Salmonella* pathogenicity island 1 (SPI-1) and SPI-2 genes. WGS-based sequence analyses facilitate a better understanding of the genetics of the antimicrobial resistance- and virulence-associated plasmids that spread among pathogens in food, animal, and clinical environments.

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers listed in Table 1 (BioProject number PRJNA312617). The SRA submission of FASTQ files has been recorded under the accession number PRJNA312617.

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