

## Supplementary Material

# Genomic analysis of two MDR isolates of *Salmonella enterica* serovar Infantis from a Spanish hospital bearing the *blaCTX-M-65* gene with and without *fosA3* in pESI-like plasmids

**Table S1.** Accession numbers of the genomes of two cefotaxime resistance isolates of *Salmonella enterica* serovar Infantis, and parameters related to the quality of the assemblies

Isolate <sup>a</sup>	kmer	Contigs	N50	Longest contig (bp)	Total bp in contigs	Contigs > 1 kb	Library	Coverage	GenBank accession number
HUMV 13-6278	93	88	245,837	1,195,887	5,000,585	44	516 +/- 130	26x	JAICDN0000000000
HUMV 15-5476	91	86	245,896	1,195,313	4,998,779	44	525 +/- 130	22x	JAICDM0000000000

<sup>a</sup>, HUMV, "Hospital Universitario Marqués de Valdecillas", Cantabria, Santander, Spain. Isolates are designated with the initials of the hospital, followed by the last two numbers of the year of recovery-serial number.

**Table S2.** Origin and accession numbers of the genomes of *Salmonella enterica* serovar Infantis isolates used for phylogenetic analysis in the present study

Isolate	Sample <sup>a</sup>	Country	Year of isolation	<i>bla</i> <sub>CTX-M</sub> / <i>fosA3</i> <sup>b</sup>	GenBank/ENA accession no. <sup>c</sup>	Reference
119944	Human feces	Israel	2008	- / -	GCA_000506925.1	[1]
MRS-16/01939	Broiler flock	Austria	2020	- / -	SAMN19328299	[2]
MRS-17/00712	Broiler flock	Austria	2020	- / -	SAMN19328300	[2]
17-01817	Human feces	Germany	2017	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702755 <sup>c</sup>	[3]
17-04725	Human feces	Germany	2017	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702767 <sup>c</sup>	[3]
18-01586	na	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX470278 <sup>c</sup>	[3]
18-01982	Human feces	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702792 <sup>c</sup>	[3]
18-02671	Human feces	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702799 <sup>c</sup>	[3]
18-03381	Human feces	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702804 <sup>c</sup>	[3]
18-04022	Human feces	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702810 <sup>c</sup>	[3]
18-06875	na	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702828*	[3]
18-06974	Human feces	Germany	2018	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702829 <sup>c</sup>	[3]
19-00103	Human feces	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702832 <sup>c</sup>	[3]
19-01785	Human feces	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702844 <sup>c</sup>	[3]
19-03124	Human feces	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702861 <sup>c</sup>	[3]
19-04093	Human feces	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702868 <sup>c</sup>	[3]
19-04501	na	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702875 <sup>c</sup>	[3]
19-04642	Human feces	Germany	2019	<i>bla</i> <sub>CTX-M-1</sub> / -	ERX4702878 <sup>c</sup>	[3]
16092401-41	Broiler chicken	Italy	2016	<i>bla</i> <sub>CTX-M-1</sub> / -	ERS2521096 <sup>c</sup>	[4]
16092401-42	Broiler chicken	Italy	2016	<i>bla</i> <sub>CTX-M-1</sub> / -	ERS2521097 <sup>c</sup>	[4]
16072017	Broiler chicken	Italy	2016	- / -	ERS2030111 <sup>c</sup>	[4]
17095712-68	Broiler meat	Italy	2017	- / -	ERS2521098 <sup>c</sup>	[4]
14026835	Human (na)	Italy	2014	<i>bla</i> <sub>CTX-M-65</sub> / +	ERR1014119 <sup>c</sup>	[5]
1091300903	Human feces	The Netherlands	2013	<i>bla</i> <sub>CTX-M-65</sub> / +	ERS2030077 <sup>c</sup>	[6]
1091400879	Human (na)	The Netherlands	2014	<i>bla</i> <sub>CTX-M-65</sub> / +	ERS2030079 <sup>c</sup>	[6]
1091401725	Human feces	The Netherlands	2014	<i>bla</i> <sub>CTX-M-65</sub> / +	ERS2030086 <sup>c</sup>	[6]

1091600414	Human (na)	The Netherlands	2016	<i>bla</i> <sub>CTX-M-65</sub> / +	ERS2030074 <sup>c</sup>	[6]
1091603069	Human (na)	The Netherlands	2017	<i>bla</i> <sub>CTX-M-65</sub> / +	ERS2030068 <sup>c</sup>	[6]
91264	Human (na)	England and Wales	2015	- / -	CP070301.1	[7]
N55391	Chicken breast	USA	2014	<i>bla</i> <sub>CTX-M-65</sub> / -	GCA_001931595.1	[8]
18TX11CB22-S1	Chicken breast	USA	2018	<i>bla</i> <sub>CTX-M-65</sub> / -	CP082538 - CP082539	[9]
CVM N18S2085	Chicken wings	USA	2018	- / -	CP052776.1	[10]
CVM N18S2198	Chicken breast	USA	2018	<i>bla</i> <sub>CTX-M-65</sub> / -	CP082521 - CP082522	[9]
CVM N19S0949	Chicken wings	USA	2019	- / -	CP052780.1	[10]
CFSAN003307	Creek water	USA	2011	- / -	GCA_002863785.1	[1]
FSIS1502169	Chicken ceca	USA	2015	<i>bla</i> <sub>CTX-M-65</sub> / +	CP016406 - CP016407	[8]
FSIS1502916	Comminuted chicken	USA	2015	<i>bla</i> <sub>CTX-M-65</sub> / +	GCA_001931575.1	[8]
CVM44454	Human (na)	USA	2014	<i>bla</i> <sub>CTX-M-65</sub> / -	GCA_001931615.1	[8]
FARPER-219	Chicken liver and spleen	Peru	2017	<i>bla</i> <sub>CTX-M-65</sub> / +	GCA_006402875.1	[11]
CFSAN103796	Cloacae swab	Trinidad and Tobago	2019	<i>bla</i> <sub>CTX-M-65</sub> / -	GCA_012957765.2	[12]
TR01	Poultry flock	Turkey	2017	- / -	CP040600.1	[13]
HUMV 13-6278	Human feces	Spain	2013	<i>bla</i> <sub>CTX-M-65</sub> / -	JAICDN0000000000	This study
HUMV 15-5476	Human feces	Spain	2015	<i>bla</i> <sub>CTX-M-65</sub> / +	JAICDM0000000000	This study

<sup>a</sup>, na, information not available.

<sup>b</sup>, -, absent.

<sup>c</sup>, The raw reads of the genome sequence were retrieved from the ENA (European Nucleotide Archive; <http://www.ebi.ac.uk/ena>) and assembled by using VelvetOptimiser.pl script implemented in the “on line” version of PLACNETw (<https://omictools.com/placnet-tool>). When two accession numbers are indicated they correspond to the chromosome and the pESI-like plasmids of the isolate.

## References

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**Table S3.** Pairwise distance matrix calculated from SNP in the genomes of two *Salmonella enterica* serovar Infantis human isolates from a Spanish hospital and 41 isolates from other sources and countries.

	1091300903	1091400879	1091401725	109160044	1091603069	119944	14026835	16072017	16092401-41	17005712-68	17040181	170404725	18-01986	18-01982	18-02671	18-03381	18-06875	18-06974	1870140823-51	19-00103	19-01785	19-03124	19-04093	19-04501	19-04642	60992401-42	91234	CFSAN003007	CFSAN103796	CVM44454	CVM N185-2085	CVM N185-2198	CVM N195-0949	FARPER-219	FSIS1502169	FSIS1502916	HUMV 13-6278	HUMV 15-5476	MRS-16/01939	MRS-17/00712	N55391	TB01	
1091300903	0	15	22	32	32	97	25	123	131	113	161	126	169	148	134	155	122	158	156	36	160	178	170	126	153	196	132	123	164	48	18	144	39	38	27	24	26	14	20	147	257	28	251
1091400879	15	0	17	27	27	94	22	120	128	110	158	123	160	145	131	152	119	155	153	31	157	175	167	123	150	193	129	120	161	43	18	141	34	33	22	19	21	11	17	144	254	18	248
1091401725	22	17	0	34	34	101	29	127	135	117	165	130	167	152	138	159	126	162	180	38	164	182	174	130	157	200	136	127	168	50	20	148	42	40	29	26	28	18	24	151	261	25	255
109160044	32	27	34	0	111	39	137	145	127	175	140	177	162	148	169	136	171	170	48	174	192	184	140	167	210	146	137	178	60	30	158	51	50	39	36	38	28	34	161	271	35	265	
1091603069	32	27	34	0	111	39	137	145	127	175	140	177	162	148	169	136	172	170	48	174	192	184	140	167	210	146	137	178	60	30	158	51	50	39	36	38	28	34	161	271	35	265	
119944	97	94	101	111	111	0	100	112	118	100	148	113	150	135	123	142	109	145	143	115	147	165	157	113	140	183	119	110	151	127	97	131	118	117	106	108	105	98	99	134	246	102	238
14026835	25	22	29	39	39	100	0	126	134	116	164	129	166	151	137	158	125	161	159	48	163	181	173	129	156	199	135	126	167	55	25	147	46	45	34	31	33	21	27	150	260	30	254
16072017	123	120	127	187	137	112	126	0	72	54	174	17	176	161	149	168	65	171	169	141	173	191	183	139	166	209	73	136	177	153	123	157	144	143	132	129	131	119	125	160	272	128	264
16092401-41	131	128	135	145	145	118	124	72	0	28	180	73	182	167	155	174	35	177	175	149	177	197	187	145	172	213	43	142	183	161	131	165	152	151	140	137	139	127	131	166	276	136	270
17-0181	113	110	117	127	127	100	116	54	28	0	162	55	164	149	137	156	19	159	157	131	161	179	171	127	154	197	29	124	165	143	113	145	134	133	122	119	121	109	115	148	260	118	252
17-04725	161	158	165	175	175	148	164	174	180	162	0	175	128	91	101	98	171	123	121	179	125	148	137	125	118	161	181	122	119	191	161	83	182	181	170	167	169	157	163	194	242	166	238
17095712-68	126	123	130	140	140	113	129	17	73	55	175	0	177	162	150	169	64	172	170	144	174	192	184	140	167	210	74	137	178	156	126	158	147	146	135	132	134	122	128	161	273	131	265
18-01586	165	160	167	177	177	150	166	176	182	164	128	177	0	115	108	122	173	39	57	181	41	57	55	127	54	77	183	124	151	193	165	111	184	183	172	169	171	159	165	196	244	168	240
18-01982	148	145	152	162	162	135	151	161	167	149	91	162	115	0	88	13	158	110	108	166	112	130	124	112	105	148	168	109	106	178	148	74	169	168	157	154	156	144	150	181	229	153	225
18-02671	134	131	138	148	148	123	137	149	155	137	101	190	108	88	0	98	96	95	152	100	118	112	100	93	136	156	97	104	164	134	84	155	154	143	140	142	130	136	169	217	139	213	
18-03381	155	152	159	169	169	142	158	168	174	156	98	169	122	13	95	0	165	117	115	173	119	137	131	119	112	158	175	116	113	185	155	81	176	175	164	161	168	151	157	188	236	161	232
18-04022	122	119	126	136	136	109	125	63	35	19	171	64	173	158	146	165	0	168	166	140	170	188	180	136	165	206	36	138	174	152	122	154	148	142	131	128	130	118	124	157	269	127	261
18-06875	158	155	162	172	145	161	171	177	159	125	172	39	110	98	117	168	0	10	176	14	52	28	122	7	50	178	119	126	188	158	106	179	178	167	164	166	154	160	191	239	165	235	
18-06974	156	155	160	170	170	143	159	169	175	157	121	170	37	108	96	115	166	10	174	12	50	26	120	5	48	176	117	124	186	156	104	177	176	165	162	154	152	158	189	237	161	233	
18TX11CB22-51	36	31	38	48	48	115	43	141	149	131	179	144	181	166	152	173	140	176	174	0	178	196	188	144	171	214	150	141	182	24	34	162	31	40	163	18	32	38	165	275	15	269	
19-00103	160	157	164	174	174	147	163	173	177	161	125	174	41	112	100	119	170	14	12	178	0	54	26	124	9	44	180	190	180	169	166	168	156	160	191	237	165	237					
19-01785	178	175	182	192	192	165	181	194	197	179	148	192	57	130	118	137	188	52	50	196	54	0	68	142	47	90	198	139	146	208	178	126	196	198	187	184	186	174	180	211	259	183	255
19-03124	170	167	174	184	184	157	173	183	187	171	137	184	55	124	112	131	180	28	26	188	26	68	0	136	23	50	190	133	140	200	170	120	191	190	179	176	178	166	170	208	251	175	249
19-04093	126	123	130	140	140	113	129	139	145	127	125	140	127	112	100	119	136	122	120	144	124	142	136	0	117	160	146	87	128	156	126	108	147	146	135	132	122	128	159	223	131	219	
19-04501	155	150	157	167	167	140	156	166	172	154	118	167	54	106	98	112	168	7	5	171	9	47	23	117	0	45	173	114	121	183	155	101	174	173	162	159	161	149	155	186	234	158	230
19-04642	196	198	200	210	210	183	199	206	213	197	161	210	77	148	136	155	206	50	48	214	44	90	50	160	45	0	216	157	164	226	196	144	217	216	205	202	204	192	194	227	273	201	273
6092401-42	132	129	136	146	146	119	135	73	43	29	181	74	183	168	156	175	36	178	176	150	180	198	190	146	173	216	0	143	184	162	132	164	153	152	141	138	140	128	134	167	279	137	271
91264	123	120	127	137	137	110	126	136	142	124	122	137	124	109	97	116	133	119	117	141	121	139	133	87	114	157	143	0	125	153	123	105	144	143	132	129	131	119	125	15			

**Table S4.** Oligonucleotides designed for this work and used for the assembly of the resistance regions (R1 and R2) of pESI-like plasmids found in *Salmonella enterica* serovar Infantis isolates from a Spanish hospital

Primer name	Sequence (5'-3')
<i>tauE</i> fam	ACCTATAACGGAACCGATGACC
IS1380 fam Flq	GGTGTGGAAATTTCGTTGAG
IS903B	AGTGGCTAATCAGCGAATGACC
<i>tonB</i>	CGATATTCAAGGTCAACGATGCC
<i>fosA3.3</i>	CAGGGATTGAATCATCTGACGC
<i>fosA3.2</i>	CAGTAAGCTGAACTAACCCGTC
<i>fosA3.1</i>	GACGGGTTAGTTCAGCTTACTG
IS91 fam.1	TGAAGTACTGCCTGAACAACCC
IS91 fam.2	GCTTCAGGTGATCAAGGATCTG
<i>floR.1</i>	ATAGAGGCTAACGTGAGTTGG
<i>floR-2</i>	CCAACTCACGTTGAGCCTCTAT
IS6 fam	GCCCTGAAATCATCCTCGAC
<i>aac(3')-IVa</i>	TCCTGAAGAATGGTGAGTGTGTC
DUF 1819 fam	AGGCAGCTTCATCAAGCTTTG
<i>dfrA14.1</i>	CAGAAGCCACTGATTGTAGGTC
<i>dfrA14.2</i>	GACCTACAATCAGTGGCTTCTG
IS3 fam	CACACCAAACACGTGCTGTATC
<i>aph(3')-Ia</i>	ACCATGAGTGACGACTGAATCC
MFS-trans-flq	GTGTACGTCGGTAATCATCTG