

Spread of *bla*_{CTX-M-9} and other clinically relevant resistance genes, such as *mcr-9* and *qnrA1*, driven by IncHI2-ST1 plasmids in clinical isolates of monophasic *Salmonella enterica* serovar Typhimurium ST34

Table S1. Accession numbers of the genomes of monophasic *Salmonella enterica* serovar Typhimurium ST34 isolates, positive for *bla*_{CTX-M-9}, from a Spanish hospital; parameters related to the quality of the assemblies as provided by VelvetOptimizer implemented in PLACNET (<https://castillo.dicom.unican.es/upload/>).

Isolate ^a	kmer	Contigs	N50	Longest contig (bp)	Total bp in contigs	Contigs > 1 kb	Library	Coverage	GenBank accession number
HUD 1/12	117	131	319,261	1,014,100	5,323,913	52	528+/-145	30x	JAICCN000000000
HUD 2/12	117	123	415,385	929,792	5,309,364	44	546+/-147	35x	JAICCM000000000
HUD 3/12	117	104	341,666	1,012,649	5,122,035	36	539+/-148	21x	JAICCL000000000
HUD 2/14	115	96	341,611	929,895	5,130,018	32	561+/-152	25x	JAICCK000000000
HUD 3/15	119	98	415,425	838,161	5,288,026	34	561+/-149	25x	JAIC CJ000000000
HUD 1/16	117	126	319,361	650,392	5,256,219	46	539+/-145	24x	JAICCI000000000
HUD 2/16	89	112	341,443	650,087	5,222,712	39	569+/-152	25x	JAICCH000000000

^a, Isolates are designated with the initials of the center which supplied them, followed by a serial number/last two numbers of the year of recovery. HUD, "Hospital Universitario de Donostia", Basque Country, Spain.

Table S2. Oligonucleotides used for the assembly of pHUD 1/16, the resistance regions of other IncHI2-ST1 plasmids, and the chromosomal RR regions found in monophasic *Salmonella enterica* serovar Typhimurium ST34 isolates from a Spanish hospital.

Primer name	Sequence (5'-3')
hp	AGATTCAGCCTGATCGCAGATTG
Tn3 fam.1	GTCGGTTTCGTTGAACGTGTAG
intI1.1	TCGCCATCACATGTATGACCAG
intI1.2	CTGGTCATACATGTGATGGCGA
intI1.3	GAGCAGCAACGATGTTACGCAG
aadB1	GAGTTACTTGACTGCGAACCTG
aadB2	CAGGTTCCGAGTCAAGTAACTC
sul1.1	TGCTAGGCATGATCTAACCCTC
sul1.2	GAGGGTTAGATCATGCCTAGCA
qacE delta1	CTCACAGCCAAACTATCAGGTC
aadA2.1	GACGCTTAGCACCTCTGATAGT
aadA2.2	ACTATCAGAGGTGCTAAGCGTC
floR.1	ATAGAGGCTCAACGTGAGTTGG
floR-2	CCAACCTCACGTTGAGCCTCTAT
ISVsa3	TGAAGTACTGCCTGAACAACCC
tnpR.1	TGCTCATCAGCTCAGTATTGCC
blaTEM1.1	AGACAGATCGCTGAGATAGGTG
hp	ACAGCTTTCTAAGAACGGTGGG
merE	CCTTCAAGGGAAGATCATGACC
merR.fl	CAAAGGGGTAAGTGACCAAACC
IS6100.1	TGCAGCGATCACCGAATTGAAG
IS6100.2	TGCAGCGATCACCGAATTGAAG
GNAT.1	CTTCATCGCAGAACGCCTAGAA
GNAT.2	TTCTAGGCGTTCTGCGATGAAG
Primer name	Sequence (5'-3')
hypA	TGCAGTGCACCTCTCCTCAACAT
ampR.1	CACTCCAGAGTATCCGCAATCC
ampR.2	GAGGTATCGAACACCATTAACCG

qnrA1.2	GCTTTGGCATAGAGTTCAGGGA
qnrA1.3	TCCCTGAACTCTATGCCAAAGC
ISCR1.1	CTCACGAGTTGTTATCAGCCAC
ISCR1.2	TCCAACACTGCTCAACACTGCC
tetA fl	GTGAATCTGCTATGGCAATCGG
tetR fl	AGGCTGTTTGCTTCTGACGAGA
drfA16.2	ATCGAGCGAGATGGAGACATAG
drfA16.3	TGTCTCCATCTCGCTCGATGTC
CTXM9.1	CACCACCAATGATATTGCGGTG
CTXM9.2	TGGTACTGCACATTGGAAAGCG
IS3000.1	GTGGATCACATCTTCCTGGATG
IS3000.2	CTTCAACTCCAGGATACTCAGC
mcr9.1	TTGCCAGAAGTAAAACCAGCGG
mcr9.2	CCGCTGGTTTTACTTCTGGCAA
mcr9.3	GAATGTGCACGTAGCGATATCG
IS5 fam.1	AGTGGCTAATCAGCGAATGACC
IS5 fam.2	AGTGGCTAATCAGCGAATGACC
IS5 fam.3	GGTCATTGCTGATTAGCCACT
pcoS	GCCATTAAGGGTGCTAATCAGG
PPR	CCATTGAATGGTCAACCCCTAC
IS90-3B fl	AGCTTCTCCCTTCCCTTGACAC
traC fl	TATGCTCAAGAAGTAGCAGCGG
parA fl	ACAATCTTCCGCTCGTACGATG
parM	CGCAGTTCGCATTATCTTTGGC
htdF fl	ACAGATTCAGCTGAAGCCTGAG
terD	TCTGTCTGAAGATGCCTCAACC
insB IS1	TGACTTTGTGTCATGCAGCTCCAC
insA IS1	GAACAGGAGGGACAGCTGATAG
Tn3 fam.2	CAATCGCTTCAGTCTGAACGAC
Tn3 fam.3	GTCGTTCAGACTGAAGCGATTG
aph (3')-Ia.1	ACCATGAGTGACGACTGAATCC
aph (3')-Ia.2	GGATTCAGTCGTCATCATGGT

IS5 fam fl	TGAGTCCGATAGTGGCAATAGC
silS	GTCCGGAATGAAGATGGAAACG
silP	GCCTGCTTTATCCTGTGTATGG
silE	AACCAAAGCCCAGCAAAGATGC
STM2760	CATCTCATTGACAAGCCGGATC
STM2755	AGATCATATCCACCACCACCTG
iroB-R	ATAGCAGTCCATACAGTGGTGG
nixA	ATGGTTCGTTGATTGGTACGGC
dcm	CTTCTATCGATGCATCCATCCC
repA	ATGTTATCGACCAGTACCGAGC
tetC	AGGCATCACTTCTTGGATAGGG
merR	ATGGAAACCGTGCTGTCTGAAC
urf2-F ⁴⁶	GGAGATCGAACTGACCGAATCGG
strB-R ⁴⁶	GGATCGTAGAACATATTGGC
strB-F ⁴⁶	ATCGTCAAGGGATTGAAACC
strA-R ⁴⁶	CCAATCGCAGATAGAAGGC
strA-F ⁴⁶	CTTGGTGATAACGGCAATTC
blaTEM-F ⁴⁷	TTGGGTGCACGAGTGGGT
blaTEM-R ⁴⁷	TAATTGTTGCCGGGAAGC

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47. Arlet, G.; A. Philippon, A. Construction by polymerase chain reaction and use of intragenic DNA probes for three main types of transferable betalactamases (TEM, SHV, CARB). *FEMS Microbiol. Lett.* **1991**, *66*, 19-25.

Table S3. SNP differences between monophasic *Salmonella enterica* serovar Typhimurium ST34 isolates from a Spanish hospital (HUD; upper similarity matrix), and between their IncHI2-ST1 plasmids (pHUD; lower similarity matrix).

	HUD 1/12	HUD 1/16	HUD 2/12	HUD 2/14	HUD 2/16	HUD 3/12	HUD 3/15
HUD 1/12	0	67	5	401	77	395	76
HUD 1/16	67	0	72	372	14	366	11
HUD 2/12	5	72	0	406	78	400	77
HUD 2/14	401	372	406	0	384	16	381
HUD 2/16	77	14	78	384	0	378	3
HUD 3/12	395	366	400	16	378	0	375
HUD 3/15	76	11	77	381	3	375	0

min: 3 max: 406

	pHUD 1/16_1	pHUD 1/12_1	pHUD 2/12_1	pHUD 2/14_1	pHUD 2/16_1	pHUD 3/12_1	pHUD 3/15_1
pHUD 1/16_1	0	40	44	9	10	9	10
pHUD 1/12_1	40	0	4	41	50	41	50
pHUD 2/12_1	44	4	0	45	50	45	50
pHUD 2/14_1	9	41	45	0	19	0	19
pHUD 2/16_1	10	50	50	19	0	19	0
pHUD 3/12_1	9	41	45	0	19	0	19
pHUD 3/15_1	10	50	50	19	0	19	0

min: 0 max: 50

Tabla S4. Conjugation frequencies of IncHI2-ST1 plasmids found in monophasic *Salmonella enterica* serovar Typhimurium ST34 isolates from a Spanish hospital.

Donor strain ^a	Transfer frequency ^b (standard deviation)	
	28 °C	37 °C
HUD 1/12	1.45×10^{-3} (0.27×10^{-3})	$\leq 2.2 \times 10^{-10}$
HUD 2/12	1.35×10^{-3} (0.06×10^{-3})	$\leq 1.4 \times 10^{-10}$
HUD 3/12	1.70×10^{-7} (0.02×10^{-7})	$\leq 1.6 \times 10^{-10}$
HUD 2/14	1.27×10^{-2} (0.69×10^{-2})	$\leq 3.2 \times 10^{-9}$
HUD 3/15	2.61×10^{-4} (0.99×10^{-4})	$\leq 3.4 \times 10^{-8}$
HUD 1/16	3.10×10^{-3} (1.10×10^{-3})	$\leq 1.1 \times 10^{-10}$
HUD 2/16	2.31×10^{-3} (0.91×10^{-3})	$\leq 5.8 \times 10^{-9}$

^a, HUD, “Hospital Universitario de Donostia”, San Sebastian, Basque Country, Spain. ^b, Conjugation frequencies are reported as the number of transconjugants per donor cell, and result from the average of two independent experiments. Selection was performed with rifampicin (recipient strain) and cefotaxime (IncHI2-ST1 plasmids).