

S1 Table. Bacterial strains, plasmids and primers used in this study.

Strain, plasmid or primer	Relevant features or sequence (5'→3')	Reference
<i>S. Typhimurium</i> (and monophasic variant) strains		
IRTA GN-3728	Rifampicin-resistant	This study [Dr. Eugenia Puentes, CZ Vaccines]
IRTA $\Delta murI$	$\Delta murI$ derivative	This study
IRTA $\Delta murI \Delta alr \Delta dadX$	$\Delta murI \Delta alr \Delta dadX$ derivative	This study
IRTA $\Delta murI \Delta alr::aph(3)-IIIa \Delta dadX$	$\Delta murI \Delta alr \Delta dadX$ kanamycin-resistant intermediate	This study
ATCC 700720 (LT2)	Wild-type strain isolated from a natural source	ATCC
ATCC 14028	Reference strain, isolated from pools of heart and liver from 4-week-old chickens	ATCC
LSP 210/18 (monophasic)	ST34, isolated from urine sample in Hospital Universitario Central de Asturias (Spain). Resistance phenotype: ampicillin, cefotaxime, streptomycin, kanamycin, sulfamethoxazole, tetracycline, trimethoprim	[Dr. M.R. Rodicio, University of Oviedo]
20220515 (monophasic)	Multi-country outbreak strain, cgMLST cluster 1, ST34, isolated from 1-year old male in Galicia (Spain). Resistance phenotype: ampicillin, streptomycin, kanamycin, sulfamethoxazole, tetracycline	[Dr. Silvia Herrera-León, Centro Nacional Microbiología]
20220258 (monophasic)	Multi-country outbreak strain, cgMLST cluster 1, ST34, isolated from 38-year old woman in Galicia (Spain). Resistance phenotype: ampicillin,	[Dr. Silvia Herrera-León, Centro

	chloramphenicol, streptomycin, kanamycin, gentamycin, sulfamethoxazole, tetracycline, trimethoprim	Nacional Microbiología]
20220191 (monophasic)	Multi-country outbreak strain, cgMLST cluster 2, ST34, isolated from 8-year old woman in Andalucía (Spain). Resistance phenotype: ampicillin, streptomycin, kanamycin, sulfamethoxazole, tetracycline	[Dr. Silvia Herrera-León, Centro Nacional Microbiología]
A130	ST313 lineage I, isolated from blood in Malawi (DT56var). Resistance phenotype: ampicillin, kanamycin, sulfamethoxazole, trimethoprim	[1], [Prof. Jay Hinton, University of Liverpool]
D23580	ST313 lineage II, isolated from blood in Malawi (Untypable). Resistance phenotype: ampicillin, chloramphenicol, streptomycin, kanamycin, sulfamethoxazole, trimethoprim	[1], [Prof. Jay Hinton, University of Liverpool]
Plasmids		
pKD13	Template plasmid containing a kanamycin/neomycin resistance cassette [<i>aph</i> -(3')-IIIa] flanked by FLP recognition target (FRT) sites	[2]
pKD46	Temperature sensitive replication (repA101ts) plasmid containing λ Red genes (<i>exo</i> , <i>bet</i> , <i>gam</i>) under arabinose-inducible promoter (P_{araB}) and an ampicillin resistant marker (<i>bla</i> _{TEM-1})	[2]
pCP20	Temperature sensitive replication (repA101ts) plasmid containing arabinose-inducible FLP	[3]

recombinase and a chloramphenicol resistance
marker (*catC*)

Primer

murIKORedFw	ACCTTCTGAACCACGTCCCACCGTGCTGGTATTTGATTCC ATTCCGGGGATCCGTCGACC
murIKORedRv	CTGGTATTTACCCAAAACGCCATTAACC GCCAGTTTTTC TGTAGGCTGGAGCTGCTTCG
murIEXTFw	ACTACTCCGCTTACCCATAC
murIEXTRv	TTTCGTCCGAGGACGTTAAG
alrKORedFw	ATTCTTTTAACAAGGAATTCAAATGCAAGCGGCAACAGTC ATTCCGGGGATCCGTCGACC
alrKORedRv	CGCCACCCGGCCCGCCGCGTATTTAATCAATATACTTCAT TGTAGGCTGGAGCTGCTTCG
alrEXTFw	GTCGCGCTTCGATAACTATG
alrEXTRv	TATCCGGCCTACAGCCAATG
dadXKORedFw	CATAACTGATAAAGGAAGTGAAATGACCCGCCCTATACA GATTCCGGGGATCCGTCGACC
dadXKORedRv	CGACGCCTTAGCCTGAATTAGGTTACGTTGTCACAAACGG TGTAGGCTGGAGCTGCTTCG
dadXEXTFw	ATACCCTACGACGATCTGAG
dadXEXTRv	GAATAAGGTCGCCTGGCAAC

ATCC, American Type Culture Collection; DT, definitive type; IRTA, Instituto de Investigación y Tecnología Agroalimentarias, Generalitat de Catalunya; LSP, Laboratorio Salud Pública Principado de Asturias; cgMLST, core genome multilocus sequence typing; ST, sequence type.

Supplementary References

1. Kingsley RA, Msefula CL, Thomson NR, Kariuki S, Holt KE, Gordon MA, et al. Epidemic multiple drug resistant Salmonella Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res* 2009;19(12):2279-87.
2. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc Natl Acad Sci U S A* 2000;97(12):6640-5.
3. Cherepanov PP, Wackernagel W. Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* 1995;158(1):9-14.