

Supplementary Tables and Figures

A global genomic analysis of *Salmonella* Concord reveals lineages with high antimicrobial resistance in Ethiopia

Wim L. Cuypers^{1,2*}, Pieter Meysman¹, François-Xavier Weill³, Rene S. Hendriksen⁴, Getenet Beyene⁵, John Wain^{6,7}, Satheesh Nair⁸, Marie A. Chattaway⁸, Blanca M. Perez-Sepulveda⁹, Pieter-Jan Ceysens¹⁰, Tessa de Block¹¹, Winnie W. Y. Lee^{8,12}, Maria Pardos de la Gandara³, Christian Kornschöber¹³, Jacob Moran-Gilad¹⁴, Kees Veldman¹⁵, Martin Cormican¹⁶, Mia Torpdahl¹⁷, Patricia I. Fields¹⁸, Tomáš Černý¹⁹, Liselotte Hardy², Bieke Tack^{2,20}, Kate C. Mellor^{21,22}, Nicholas Thomson^{21,22}, Gordon Dougan²³, Stijn Deborggraeve²⁴, Jan Jacobs^{2,20}, Kris Laukens¹, Sandra Van Puyvelde^{22, 23, 25*}

¹Adrem Data Lab, Department of Computer Science, University of Antwerp, Antwerp, Belgium

²Tropical Bacteriology Unit, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

³Institut Pasteur, Université Paris Cité, Unité des bactéries pathogènes entériques, F-75015, Paris, France

⁴Technical University of Denmark, National Food Institute (DTU-Food), Research Group of Global Capacity Building, Kgs., Lyngby, Denmark

⁵Department of Medical Laboratory Sciences, Faculty of Health Sciences, Jimma University, Jimma, Ethiopia

⁶Quadram Institute Bioscience, Norwich Research Park, Norwich, UK

⁷Norwich Medical School, University of East Anglia, Norwich, UK

⁸Gastrointestinal Bacterial Reference Unit, United Kingdom Health Security Agency, Colindale, London, UK

⁹Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, Liverpool, UK

¹⁰Division of Human Bacterial Diseases, Sciensano, Brussels, Belgium

¹¹Clinical Reference Laboratory, Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

¹²MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, UK

¹³Austrian Agency for Health and Food Safety (AGES), Institute for Medical Microbiology and Hygiene, 8010 Graz, Austria

¹⁴Department of Health Policy and Management, School of Public Health, Faculty of Health Sciences, Ben Gurion University of the Negev, Israel

¹⁵Department of Bacteriology, Host Pathogen Interaction & Diagnostics, Wageningen Bioveterinary Research, Lelystad, The Netherlands

¹⁶Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Galway, Ireland

¹⁷Department of Bacteriology, Mycology & Parasitology, Statens Serum Institut, 5 Artillerivej, DK-2300 Copenhagen S, Denmark

¹⁸Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

¹⁹National Reference Laboratory for salmonella, State Veterinary Institute Prague, Prague, Czech Republic

²⁰Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

²¹London School of Hygiene and Tropical Medicine, Bloomsbury, London, UK

²²Wellcome Trust Sanger Institute, Genome Campus, Hinxton, Cambridge, United Kingdom

²³Cambridge Institute of Therapeutic Immunology & Infectious Disease (CITIID), Department of Medicine, University of Cambridge, Cambridge CB2 0SP, United Kingdom

²⁴Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium

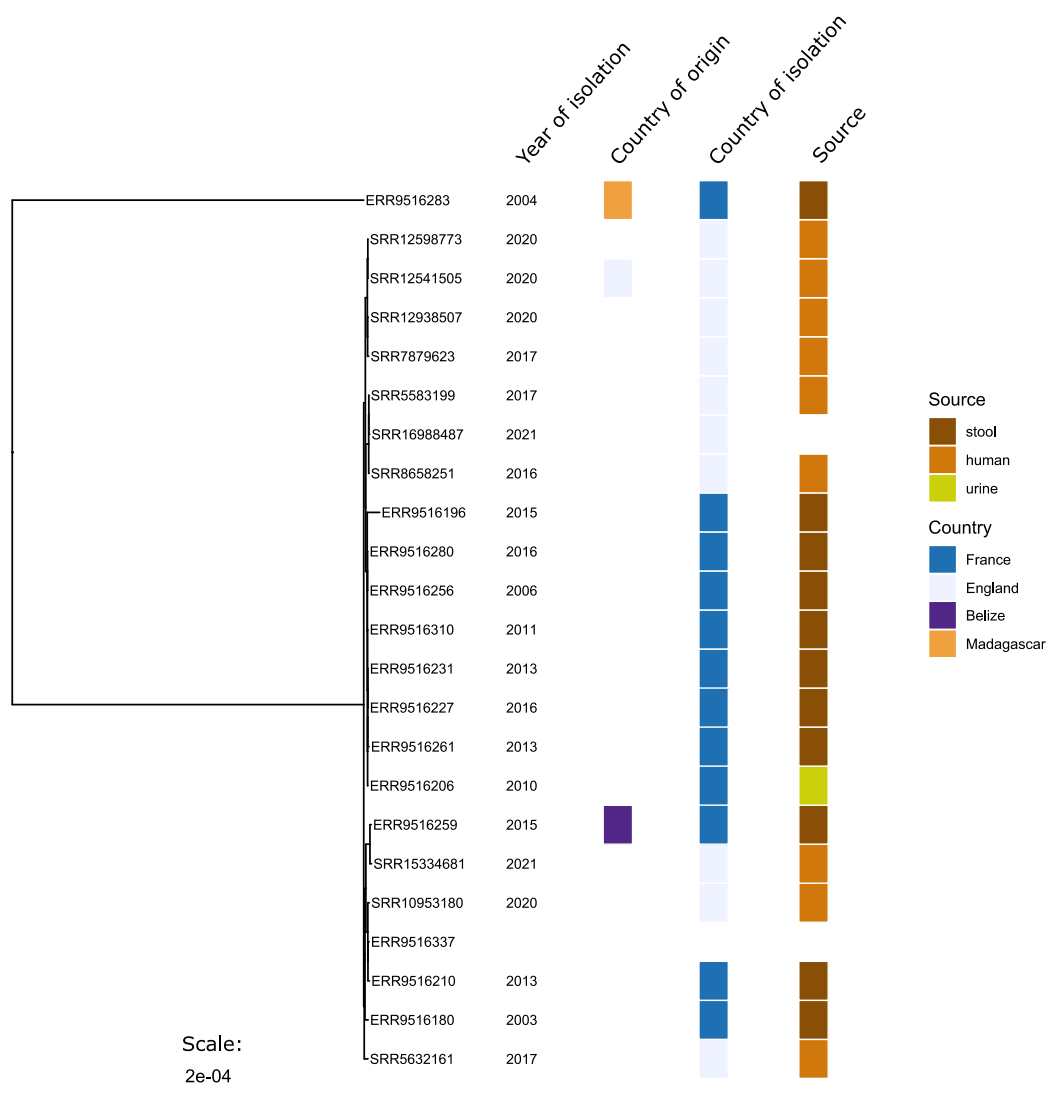
²⁵Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium

*Correspondence should be addressed to W.L.C. and S.V.P. (email: wim.cuypers@uantwerpen.be and sandra.VanPuyvelde@uantwerpen.be)

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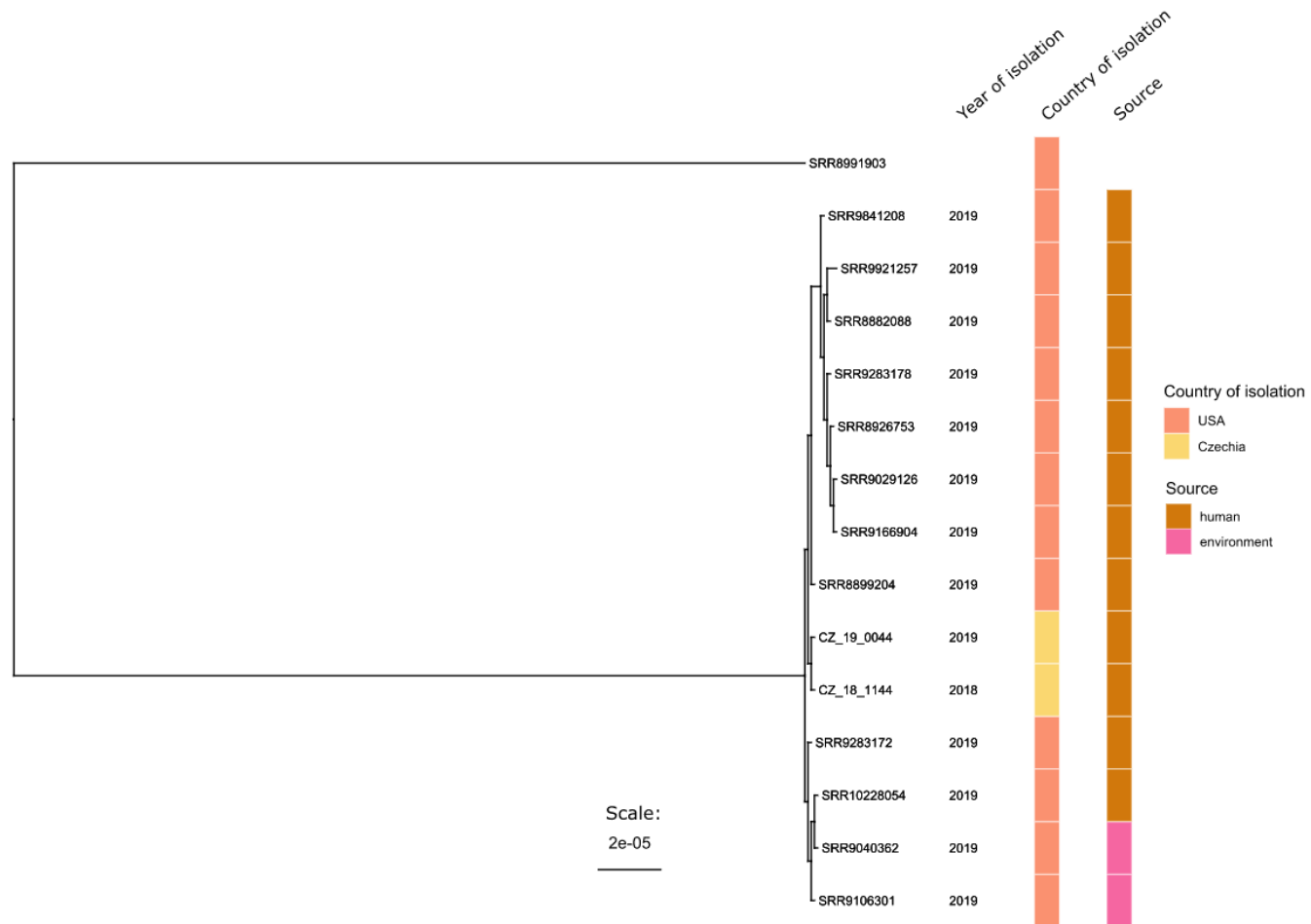
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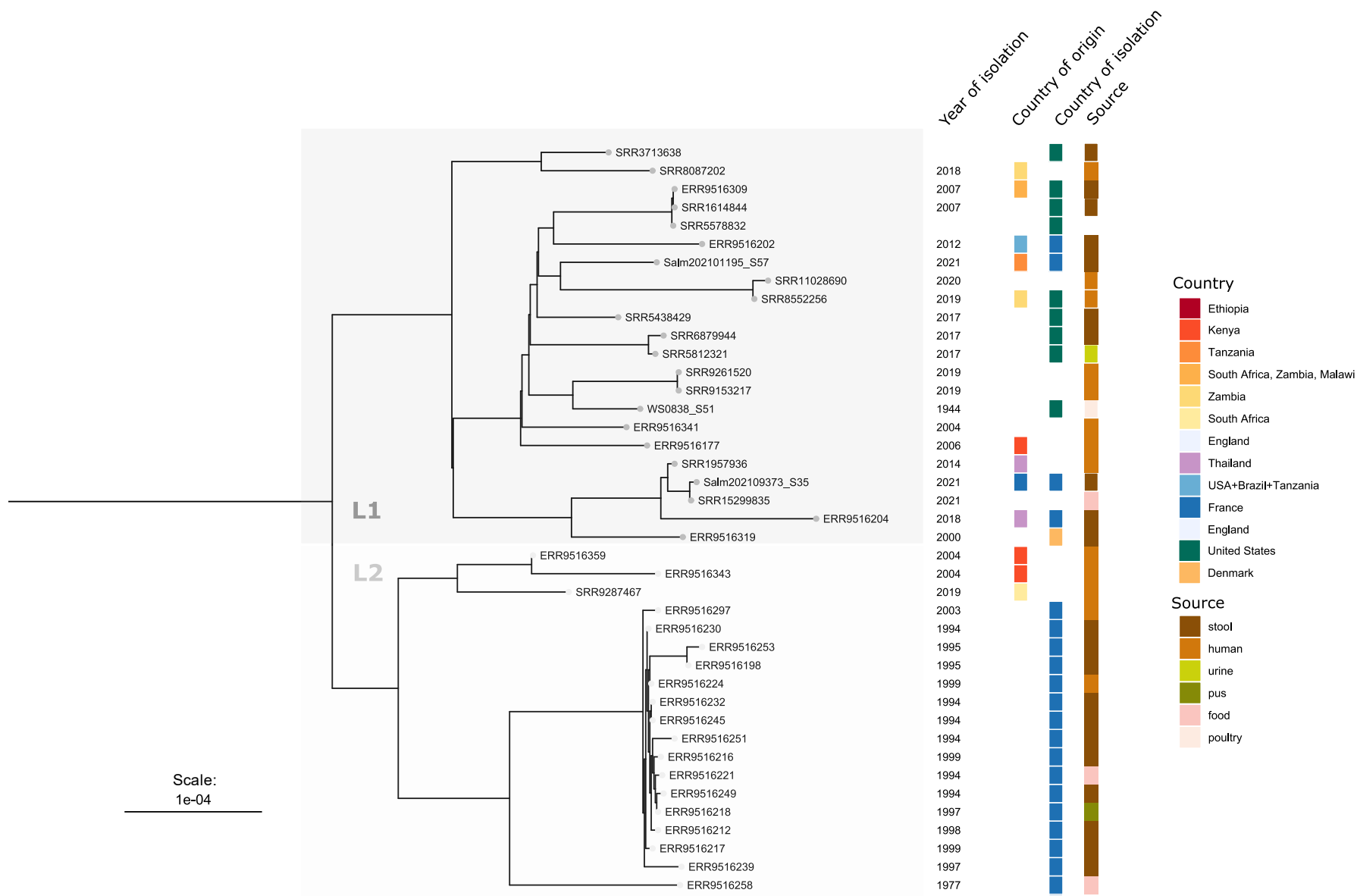
Supplementary Figure 1: S. Concord super-lineage B (HC2000_141)

Core gene maximum likelihood tree of S. Concord super-lineage B isolates. Leaves show the accession number. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of origin, country of isolation and isolation source).



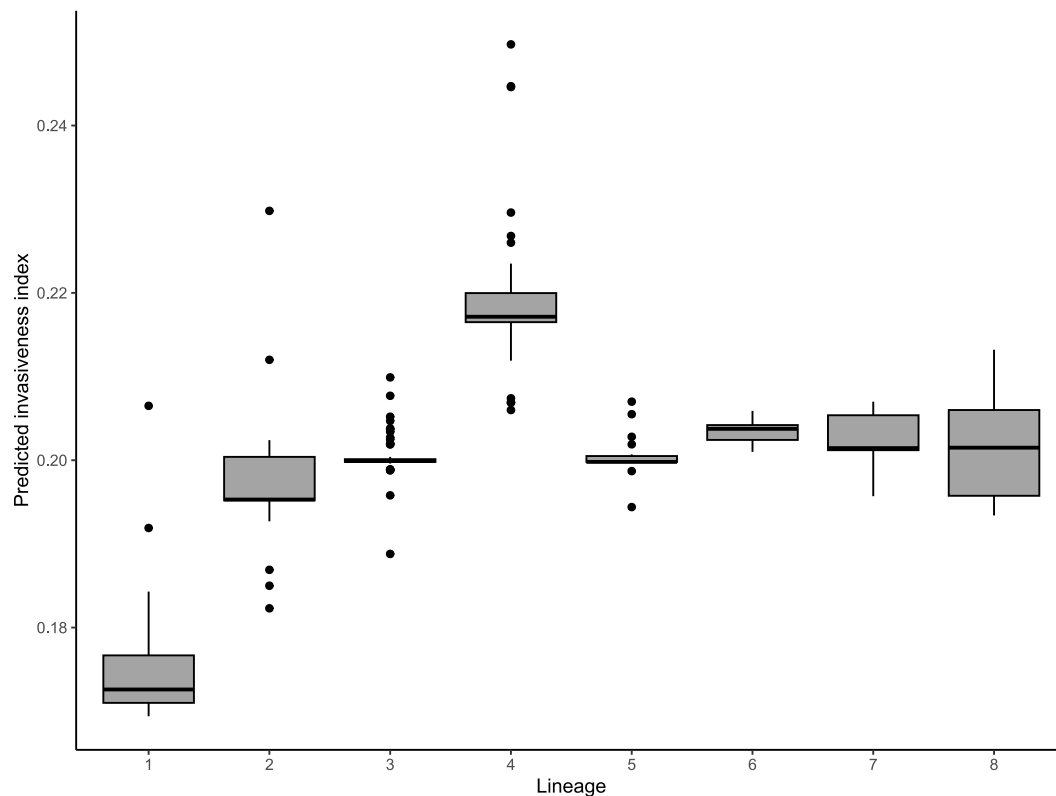
Supplementary Figure 2: *S. Concord* super-lineage C (HC2000_177997)

Core gene maximum likelihood tree of *S. Concord* super-lineage C. Leaves show the accession number or isolate ID. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of isolation and isolation source).



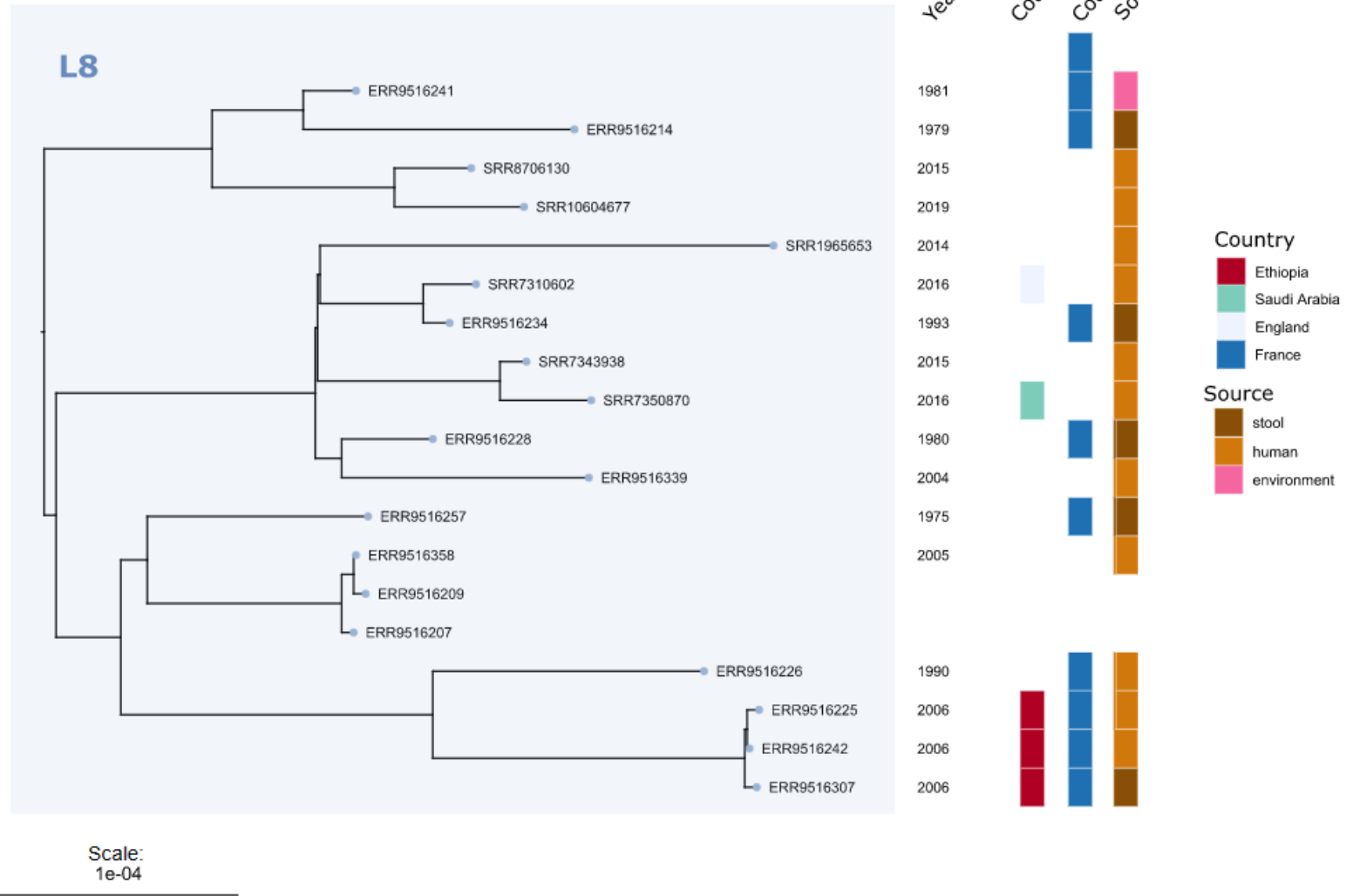
Supplementary Figure 3: Detailed view of S. Concord super-lineage A lineages 1 (L1) and 2 (L2)

Leaves show the accession number of each isolate. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of origin, country of isolation and isolation source).



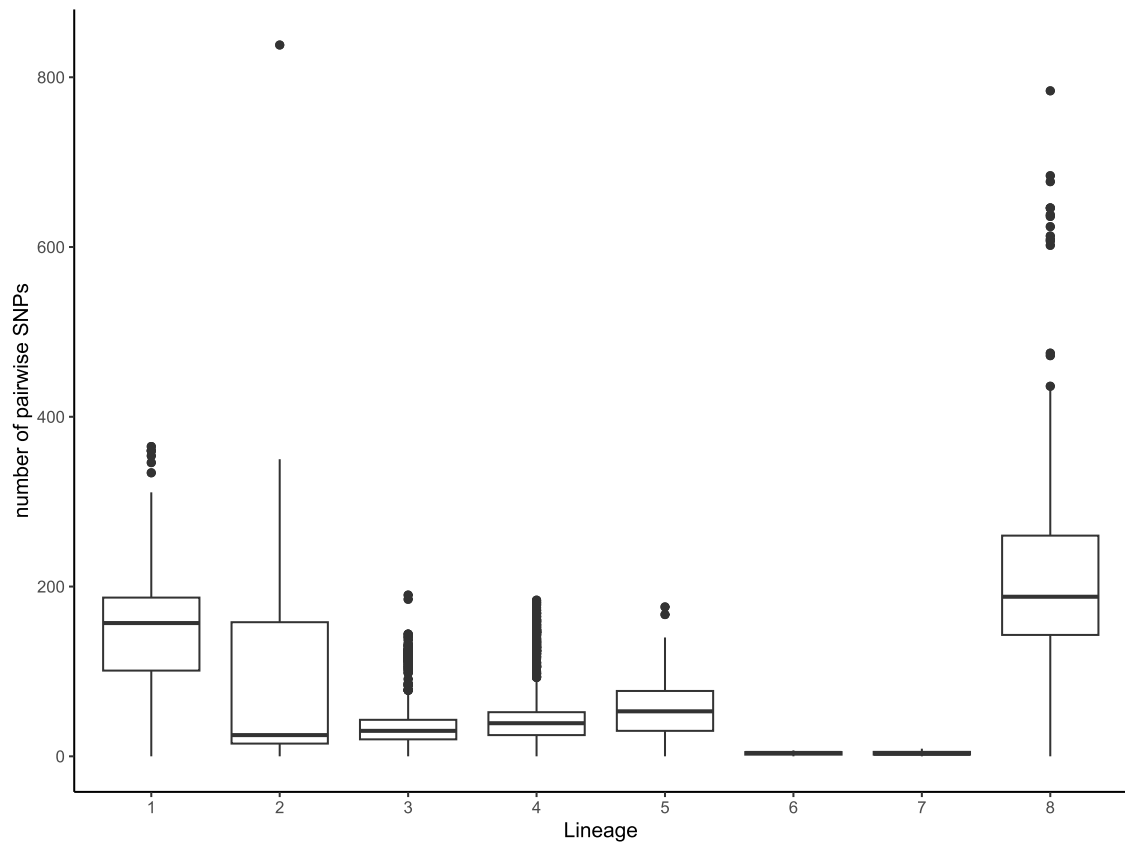
Supplementary Figure 4: Invasiveness index predicted for all *S. Concord* Super-lineage A isolates based on genomic features

Boxplot showing the distribution of the invasiveness index, as predicted per isolate by Wheeler et al.'s methodology (2018)¹. Invasiveness index values (y-axis) were grouped per lineage (x-axis). Horizontal lines in each box represent the median value of the distribution, while the upper and lower quartiles are represented by the box limits. The whiskers extend to 1.5 times the interquartile range and individual data points beyond this range are considered outliers. The invasiveness index was predicted using a publicly available pipeline (https://github.com/Gardner-BinfLab/invasive_salmonella/tree/master/fastq_pipeline) that maps reads for each isolate onto a set of 196 genes previously linked to increased invasiveness, and generates a consensus protein sequence from the mapped reads for comparison to the same 196 genes using a functional variant calling metric². The resulting features are then used as input for a pre-trained random forest classifier. The proportion of decision trees classifying an isolate as 'invasive' is referred to as the invasiveness index. Source data are provided as a Source Data file.



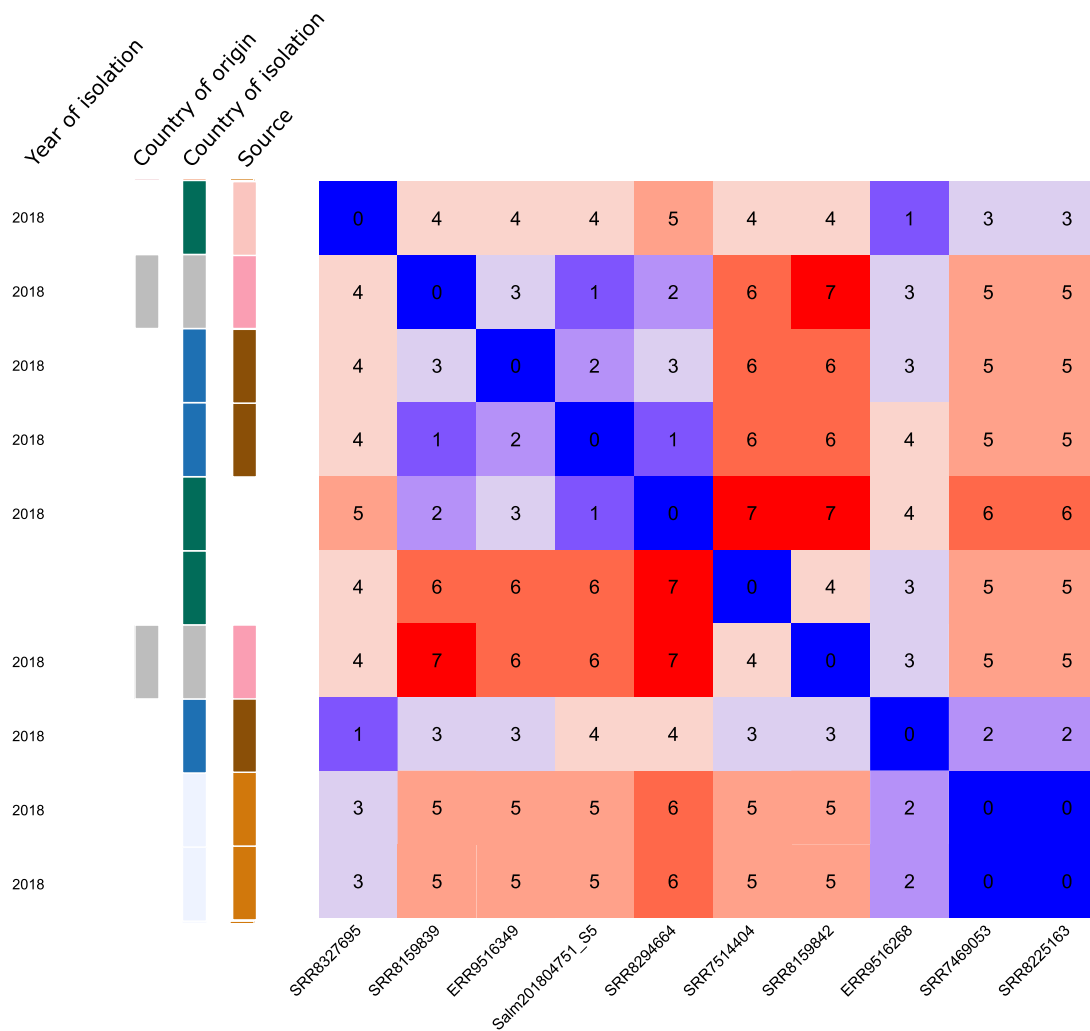
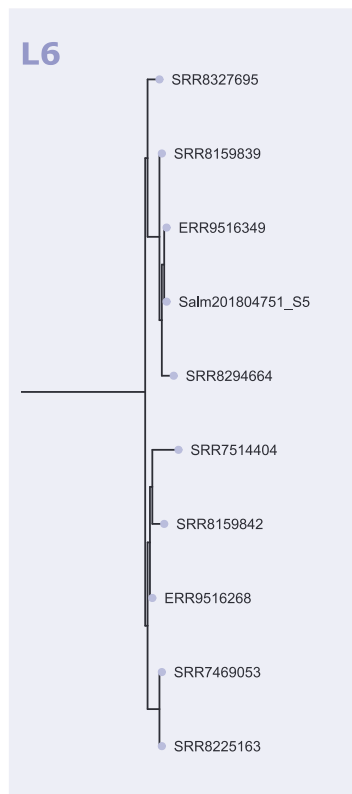
Supplementary Figure 5: Detail of super-lineage A lineage 8 (L8) showing the mixed occurrence of recent and historical isolates

Leaves show the accession number of each isolate. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of origin, country of isolation and isolation source).



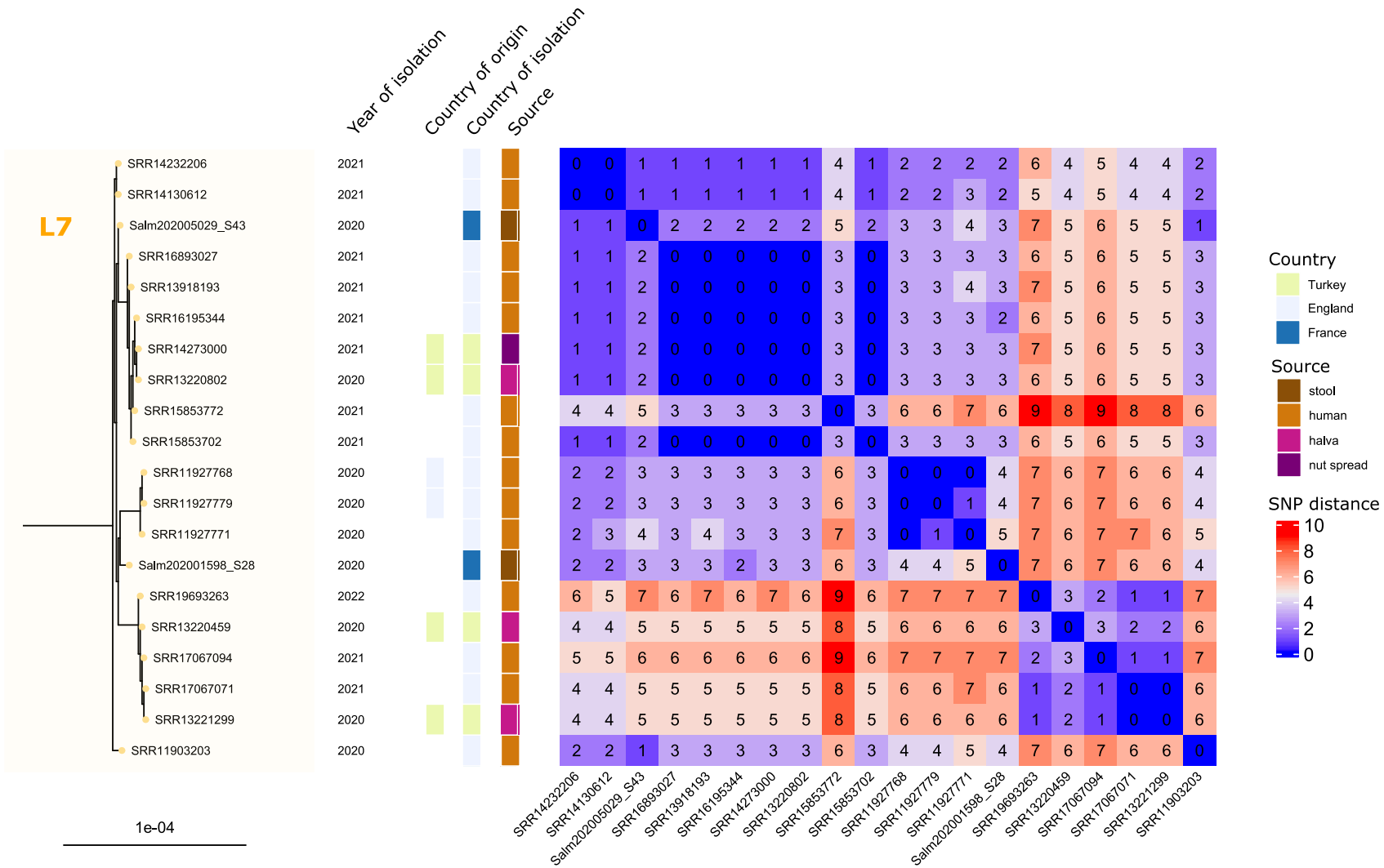
Supplementary Figure 6: Pairwise SNP differences within each *S. Concord Super-lineage A* lineage

Boxplot showing the distribution of pairwise SNP differences between all isolates within a given lineage. Pairwise SNP differences (y-axis) were calculated using `snp-dists`³ v0.7.0 from the core SNP alignment used for phylogenetic inference prior to removing recombinant regions. Values were subsequently grouped per lineage (x-axis). Horizontal lines in each box represent the median value of the distribution, while the upper and lower quartiles are represented by the box limits. The whiskers extend to 1.5 times the interquartile range and individual data points beyond this range are considered outliers. Source data are provided as a Source Data file.



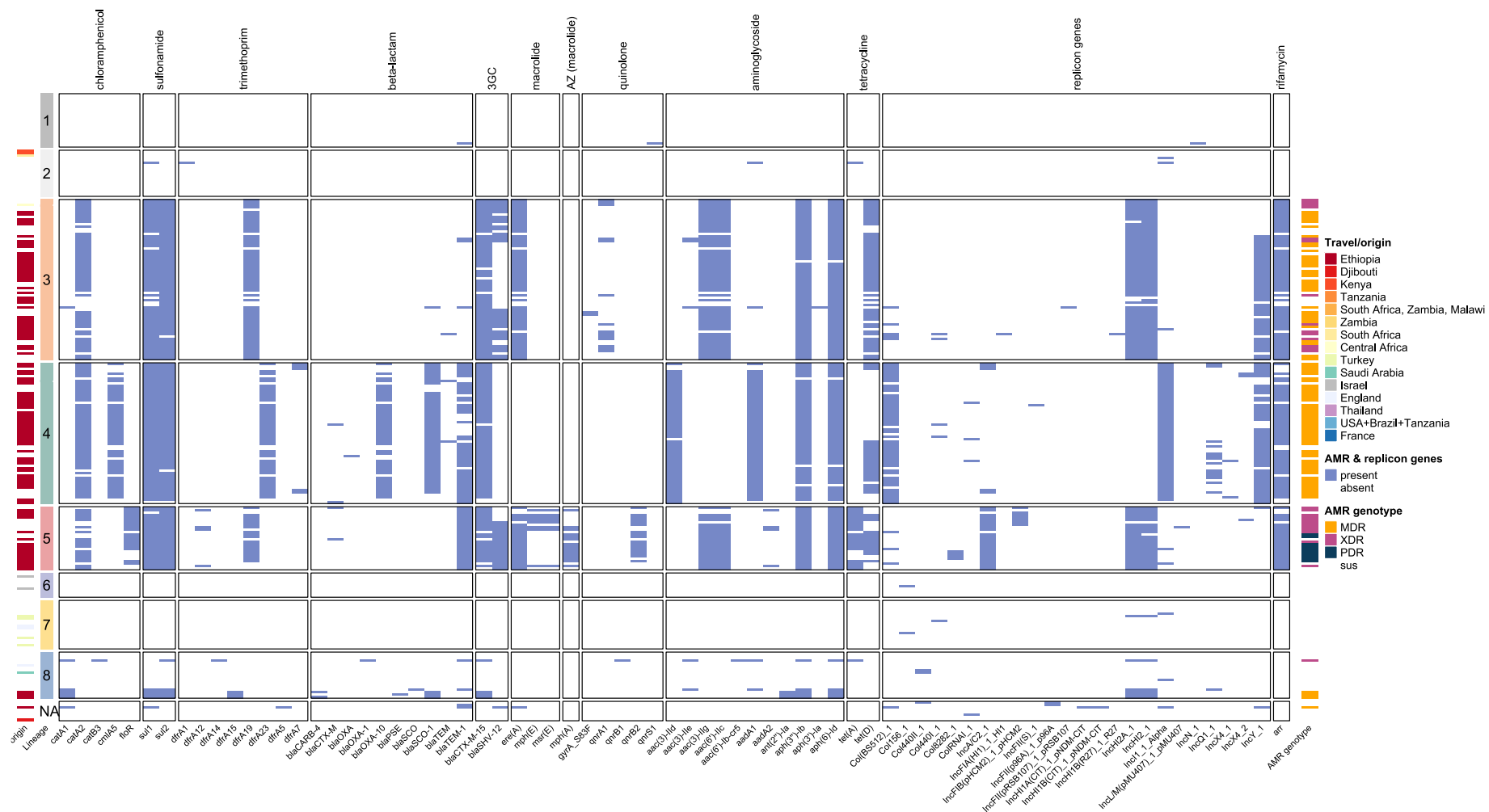
Supplementary Figure 7: Detail of super-lineage A lineage 6 (L6) showing the close relatedness of isolates from Tahini isolated in Israel and clinical isolates

Leaves show the accession number of each isolate. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of origin, country of isolation and isolation source). The heatmap on the right-hand side of the figure shows the SNP distance between all isolates



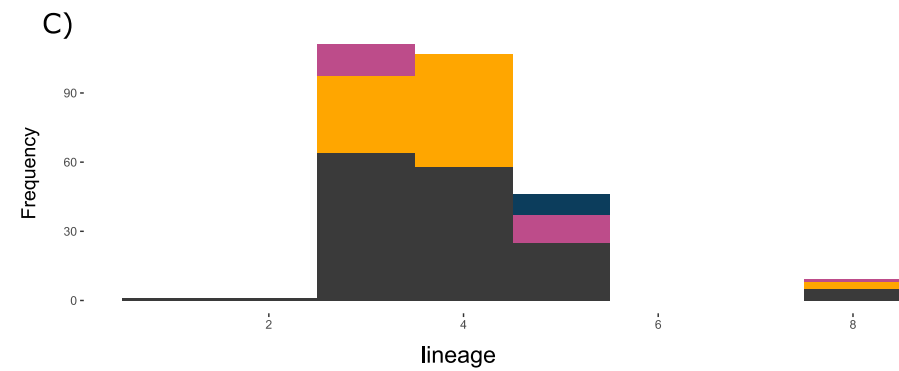
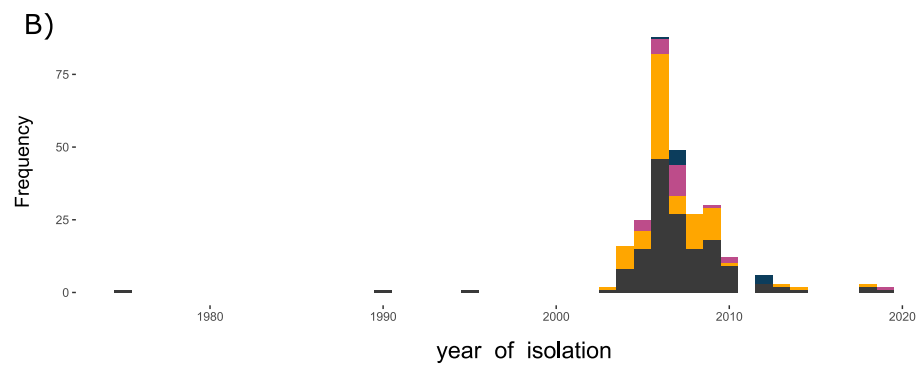
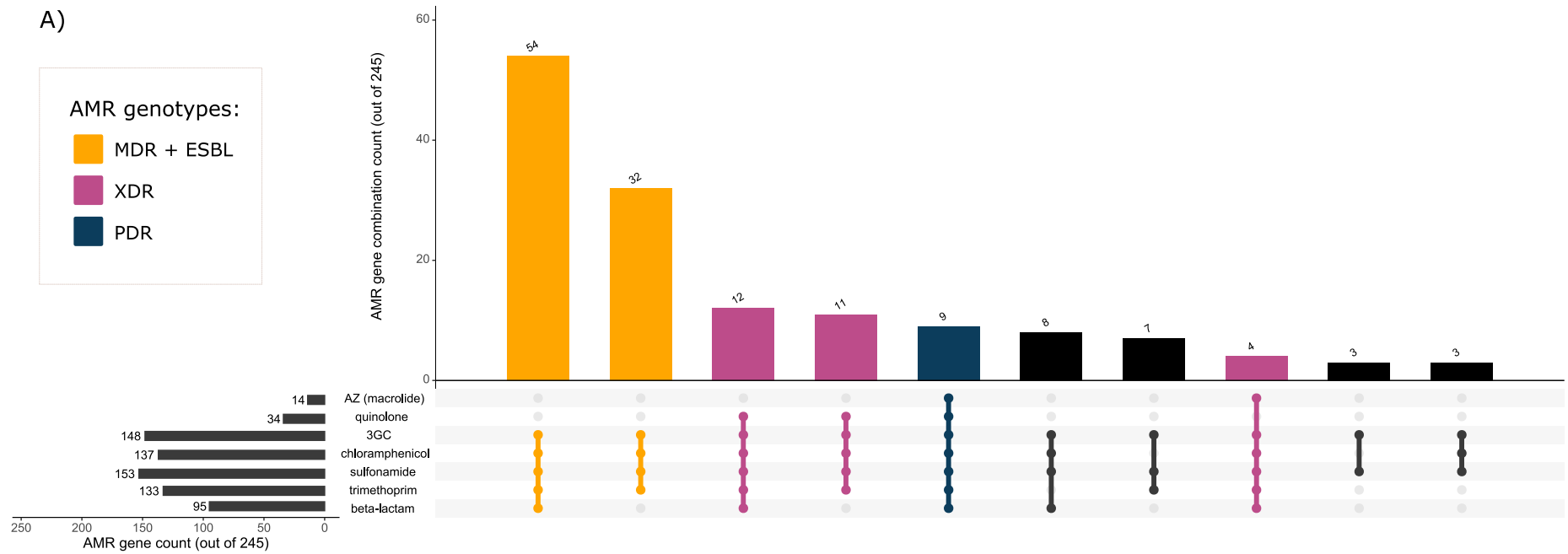
Supplementary Figure 8: Close relatedness of clinical isolates and isolates from Halva food products from Turkey in super-lineage A lineage 7 (L7)

Leaves show the accession number of each isolate. Complementary metadata is shown as text (year of isolation) or as a coloured track (country of origin, country of isolation and isolation source). The heatmap on the right-hand side of the figure shows the SNP distance between all isolates.



Supplementary Figure 9: Complete set of AMR genes and plasmid replicon genes identified in *S. Concord* draft genomes, including AMR genes that do not contribute to the MDR, XDR and PDR phenotypes and less common replicon types

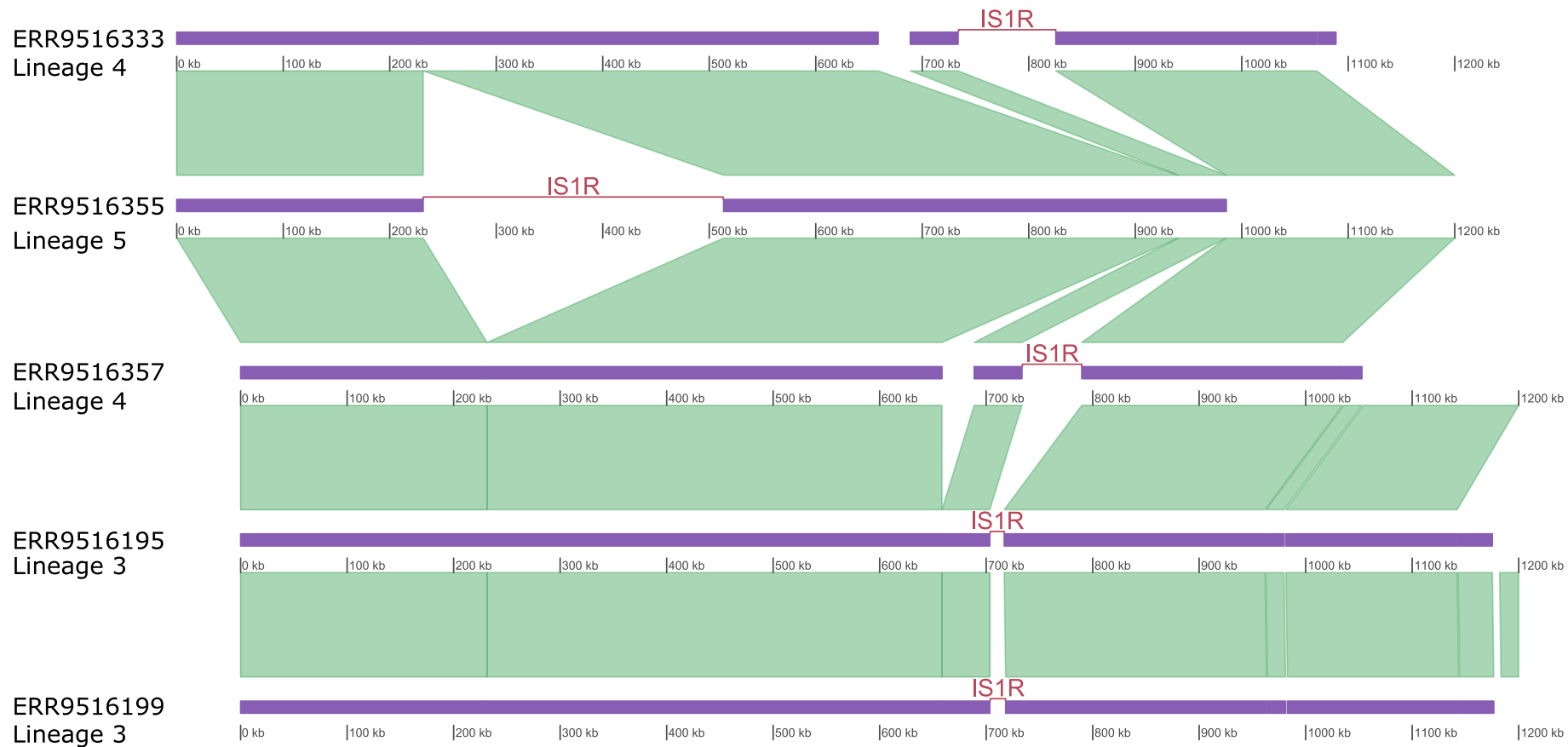
Presence (blue cells) or absence (white cells) of AMR genes identified in each *S. Concord* draft genomes using the AMRFinderPlus⁴ tool is shown (only exact matches are shown and the default database was used). Lineages are shown at the left-hand side next to the heatmap, as well as the origin of the isolate. At the right-hand side, the genomic AMR combinations are shown (MDR, XDR and PDR). The ComplexHeatmap⁵ package v2.8.0 for R was used to draw the heatmap. **Abbreviations:** AMR = antimicrobial resistance, MDR = multidrug resistance, ESBL = extended-spectrum beta-lactamase, XDR = extensive drug resistance, PDR = pandrug resistance, sus = susceptible, 3GC = third-generation cephalosporin, AZ = azithromycin. Raw data is included in **Supplementary Data 1**.



Supplementary Figure 10: Genomic AMR combinations in *S. Concord* super-lineage A

- A) UpSet plot showing the different genomic AMR combinations in the entire *S. Concord* SLA dataset. The number of resistance genes linked to a certain antimicrobial category is shown in the left, horizontally oriented barplot. The vertically oriented bar plot shows the number of times specific AMR gene combinations were present encoding MDR, XDR and PDR.
- B) Stacked barplot showing the frequency of the most common AMR gene combinations per year of isolation of the isolates. Combinations that are not classified as MDR + ESBL, XDR or PDR are shown in grey.
- C) Stacked barplot showing the frequency of the most common AMR gene combinations per lineage. Combinations that are not classified as MDR + ESBL, XDR or PDR are shown in black.

The UpSetR⁶ package v1.4.0 was used in R to construct the plots. **Abbreviations:** AMR = antimicrobial resistance, MDR = multidrug resistance, ESBL = extended-spectrum beta-lactamase, XDR = extensive drug resistance and PDR = pandrug resistance, AZ = azithromycin, 3GC = third-generation cephalosporin.

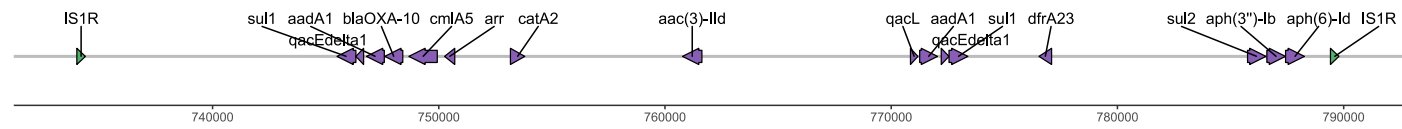


Supplementary Figure 11: Chromosomal AMR cassettes flanked by *IS1R* were integrated at different positions in the long read sequenced *S. Concord* isolates and varied in length

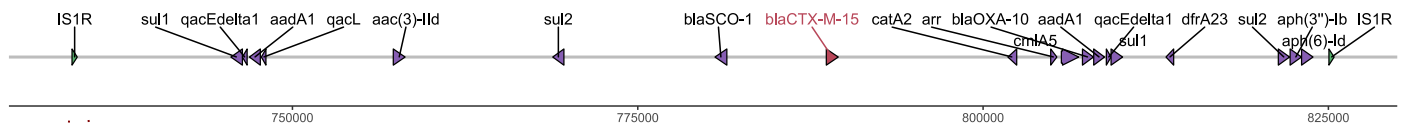
Homologous regions conserved across the genomes were determined using Mauve⁷ and visualised using the genoplotR⁸ package v0.8.11 in R. Only the first 1200 kilobases of long read sequenced chromosomes containing antimicrobial resistance (AMR) genes integrated into the chromosome are shown. Purple blocks represent conserved sequences which are connected with green lines to visualize relative changes in position. The location of a chromosomally integrated AMR gene cassette flanked by *IS1R* transposase genes is highlighted in red. On the left-hand side the accession number of each isolate is shown in addition to the lineage were the isolate clusters.

Lineage 4

ERR9516357

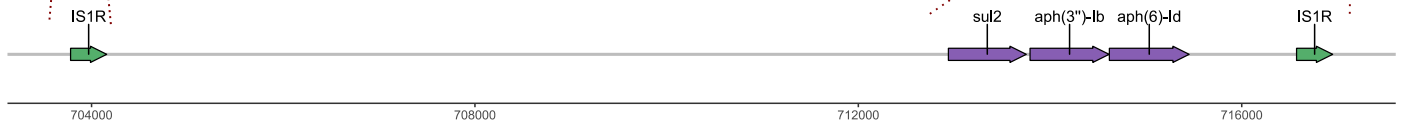


ERR9516333

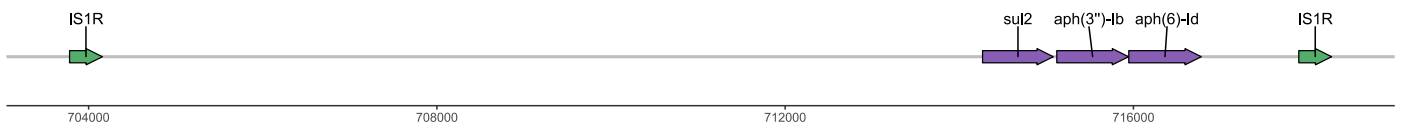


Lineage 3

ERR9516195

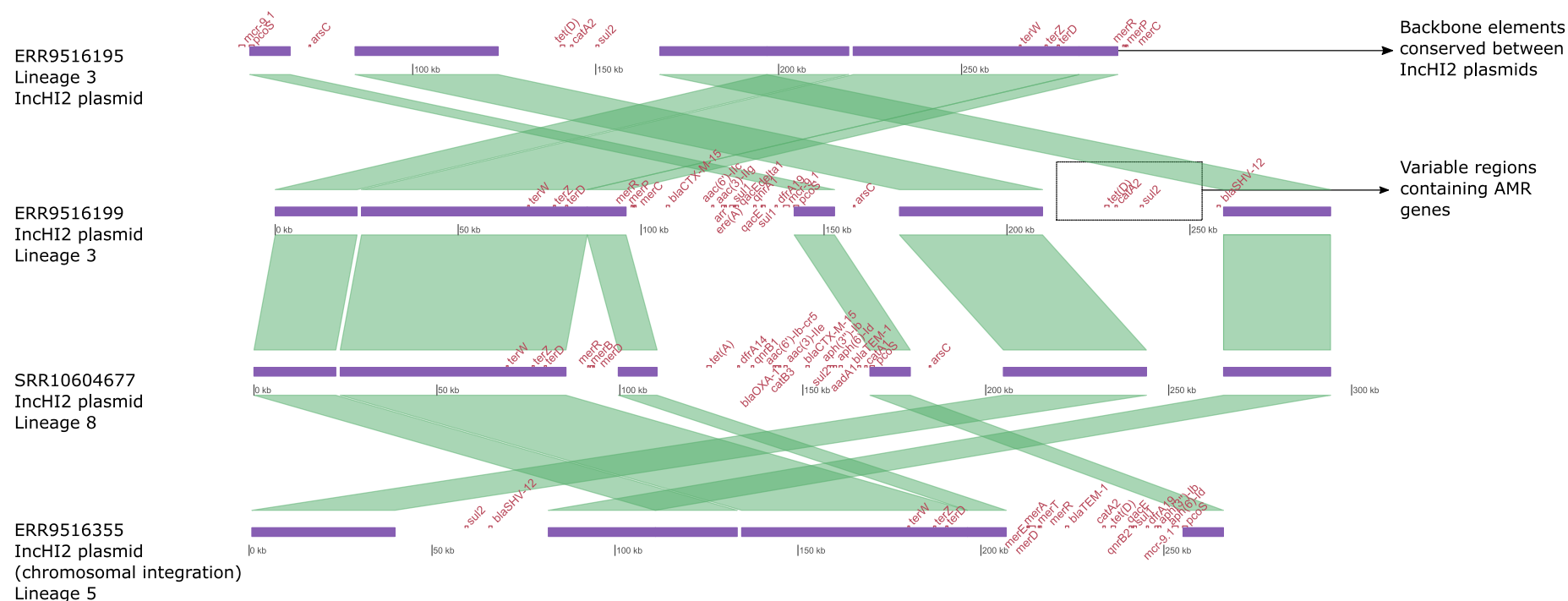


ERR9516199



Supplementary Figure 12: Chromosomal AMR cassettes flanked by IS1R varied in composition in lineages 3 and 4

Detailed view of the AMR genes integrated into the *S. Concord* chromosome in reference genomes obtained from four L3 and L4 isolates. The ESBL gene *bla_{CTX-M-15}* is highlighted in red. Other genes are linked to multidrug resistance and aminoglycoside resistance. Images were made using the R packages *ggtree*⁹ and *gggenes*¹⁰ and were combined and annotated in Inkscape v0.92.



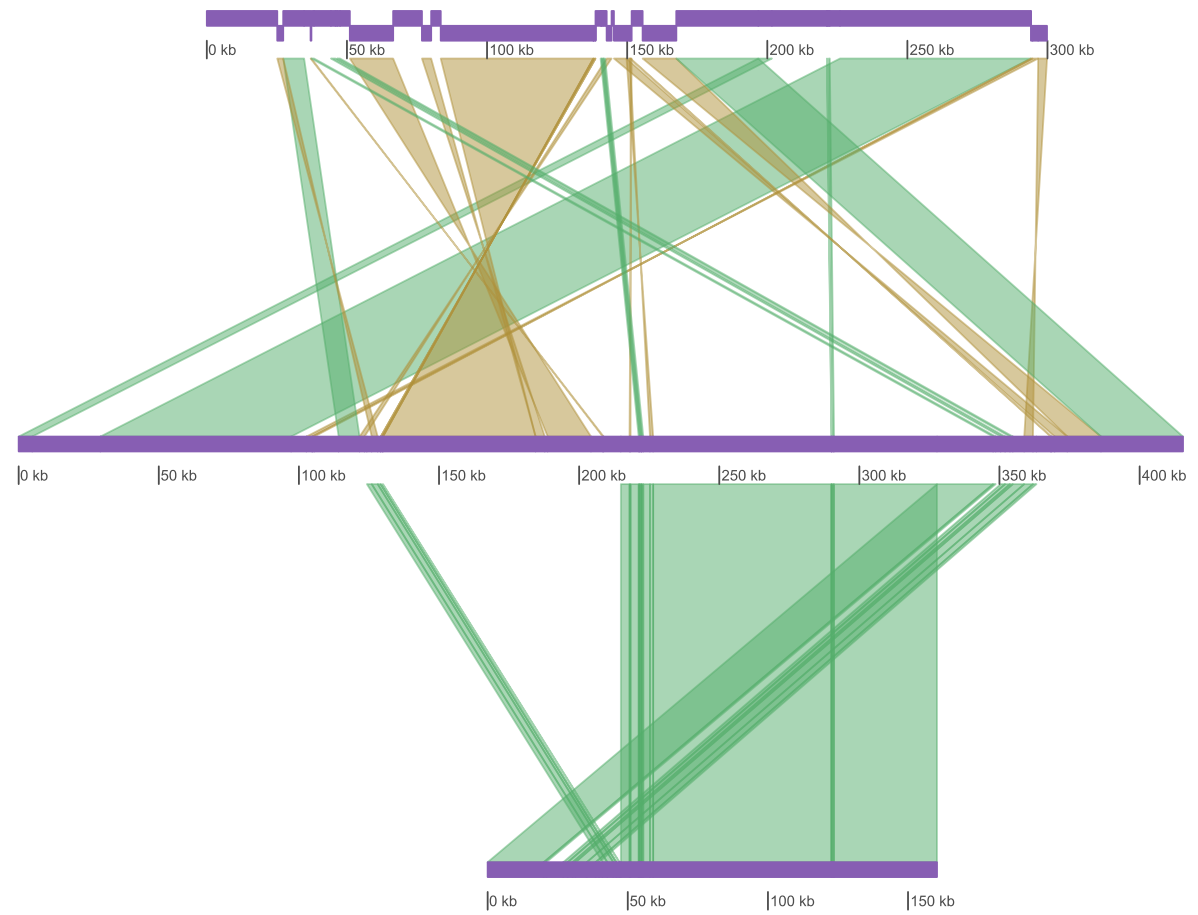
Supplementary Figure 13: IncHI2 plasmids and a chromosomally integrated IncHI2 plasmid shared common backbone elements and differed in regions encoding AMR

The alignment was made using mauve⁷ and visualised using the genoplots⁸ package v0.8.11 in R. Text annotations in red are antimicrobial resistance genes and metal resistance genes identified using AMRFinderPlus¹¹ (default database).

ERR9516195
InchI2 plasmid
Lineage 3

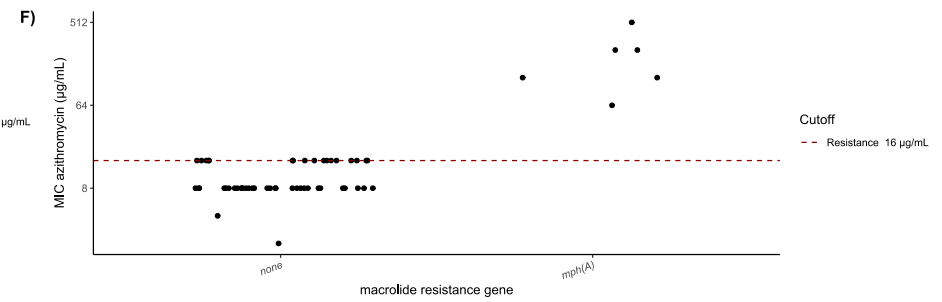
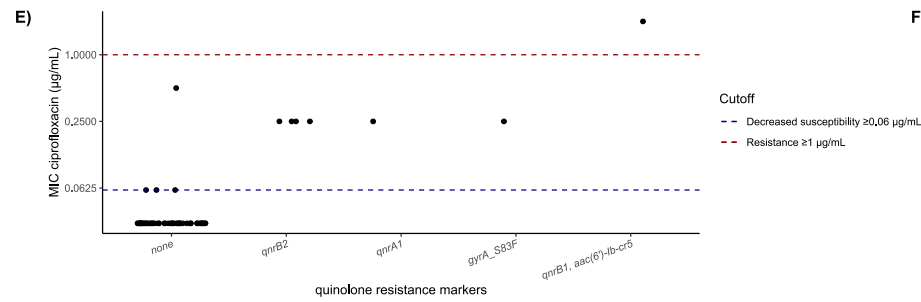
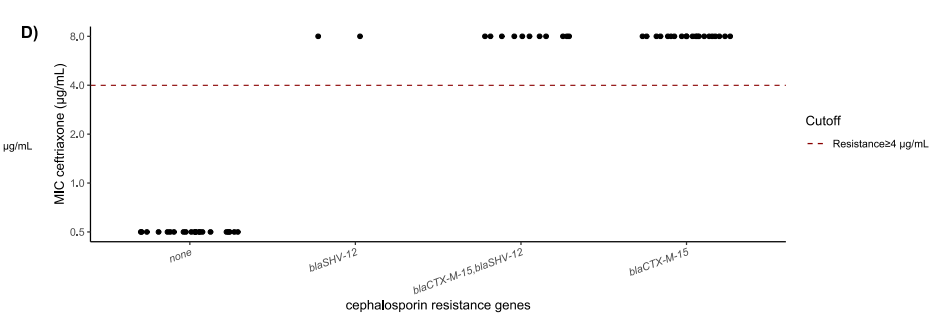
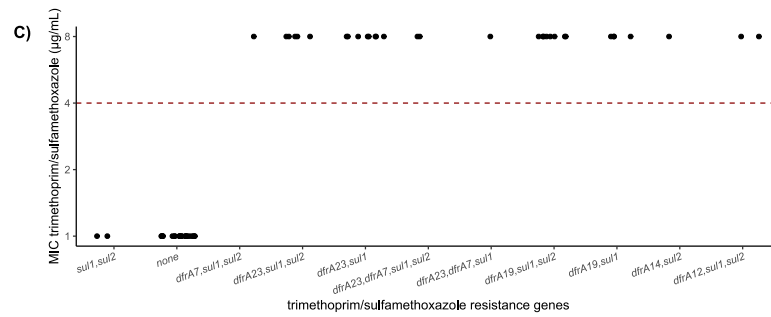
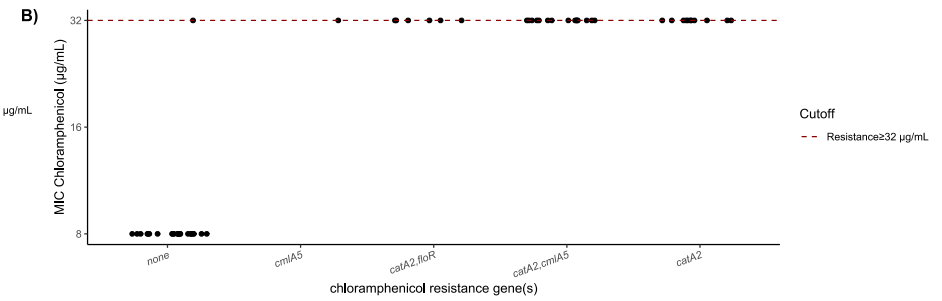
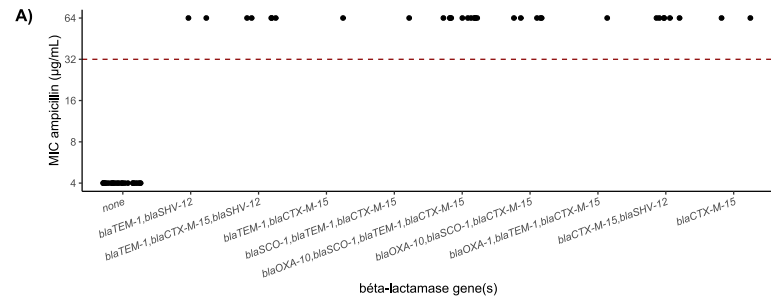
ERR9516323
InchI2/IncA/C hybrid plasmid
Lineage 5

ERR9516355
IncA/C plasmid
Lineage 5



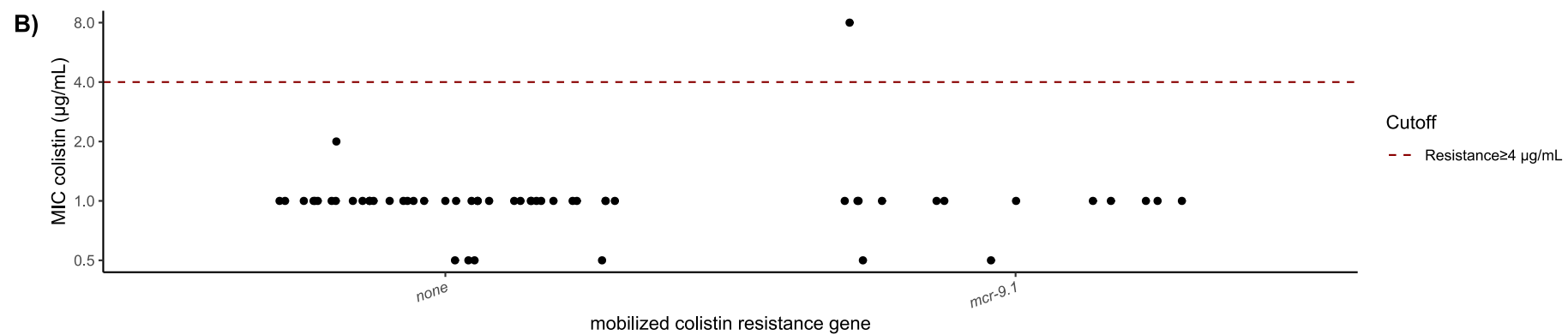
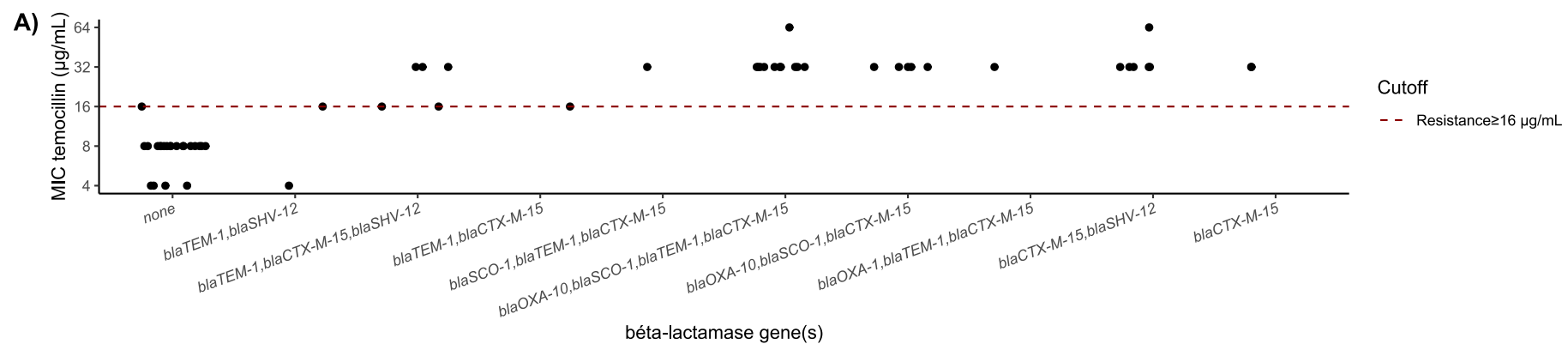
Supplementary Figure 14: The XDR hybrid plasmid identified in isolate 32640_1_296 (middle track; part of Lineage 5) reference genome contained backbone elements derived from IncHI2 and IncA/C plasmids

The alignment was made using mauve⁷ and visualised using the genoplots⁸ package v0.8.11 in R.



Supplementary Figure 15: Association of AMR genotype and phenotype for first- and second-line antimicrobials recommended to treat invasive *Salmonella* infections

Genotype-phenotype association for ampicillin (A), chloramphenicol (B), trimethoprim-sulfamethoxazole (C), ceftriaxone (D), ciprofloxacin (E) and azithromycin (D). Dots indicate the minimum inhibitory concentration (MIC) (y-axis) for each of 56 isolates harbouring a certain combination of genomic resistance markers (shown on the x- axis). The dashed line in red indicates the CLSI M100-ED31 breakpoint for clinical resistance. For azithromycin the epidemiological cutoff was indicated by the dashed line in red. For ciprofloxacin, the dashed line in **blue** indicates the decreased ciprofloxacin susceptibility cutoff. Tested concentration ranges and measurements are listed in **Supplementary Data 2**.



Supplementary Figure 16: Association of AMR genotype and phenotype for alternative antimicrobials

Genotype-phenotype association for temocillin (A) and colistin (B). Dots indicate the minimum inhibitory concentration (MIC) (y-axis) for each of 56 isolates harbouring a certain combination of genomic resistance markers (shown on the x- axis). The dashed line in red indicates the EFSA epidemiological cutoff for temocillin and the CLSI M100-ED31 breakpoint for clinical resistance for colistin. Tested concentration ranges and measurements are listed in **Supplementary Data 2**.

Supplementary Tables

Supplementary Table 1: Presence-absence variations in genes linked to increased invasive potential in L4 isolates. The number of genes that were missing (*i.e.* no significant BLAST hit) from at least one isolate from lineage 4 (L4) were reported. The full dataset was generated by comparing all annotated genes in the *S. Concord* draft assemblies to the set of 196 genes predictive of an invasive phenotype utilized in the machine learning approach by Wheeler et al. (2018), using BLAST^{12,13} v2.13.0+. Per query sequence, the hit with the highest bit score was retained, and we jointly assessed the query coverage and percentage identity by calculating their product and multiplying by 100. Source data are provided as a Source Data file.

ID	Gene	Number of L4 isolates missing the gene	Full gene name
STM1154	<i>mdtG</i>	12	Multidrug resistance protein MdtG
STM2245	-	12	Putative outer membrane protein
STM1349	<i>pps</i>	8	Phosphoenolpyruvate synthase [EC=2.7.9.2]
STM1338	<i>pheT</i>	5	Phenylalanine--tRNA ligase beta subunit [EC=6.1.1.20]
STM2066	<i>sopA</i>	5	E3 ubiquitin-protein ligase SopA [EC=6.3.2.-]
STM3152	<i>mcpB</i>	5	Putative methyl-accepting chemotaxis protein
SG1259	-	3	Putative uncharacterized protein
STM2808	<i>nrdF</i>	3	Ribonucleoside-diphosphate reductase 2 subunit beta [EC=1.17.4.1]
SEN3623	<i>yidE</i>	1	Putative transport protein YidE
STM0631	<i>ybeM</i>	1	Putative hydrolase
STM0994	<i>mukB</i>	1	Chromosome partition protein MukB
STM1746.S	<i>oppA</i>	1	Periplasmic oligopeptide-binding protein
STM1914	<i>flhB</i>	1	Flagellar biosynthetic protein FlhB
STM2036	<i>pocR</i>	1	Regulatory protein PocR
STM2043	<i>pduG</i>	1	Propanediol utilization diol dehydratase reactivation protein
STM2053	<i>pduS</i>	1	Propanediol utilization protein
STM2057	<i>pduW</i>	1	Probable propionate kinase [EC=2.7.2.15]
STM2065	<i>phsA</i>	1	Thiosulfate reductase [EC=1.-.-.]
STM2481	<i>acrD</i>	1	RND family aminoglycoside/multidrug efflux pump
STM2815	<i>emrB</i>	1	Putative MFS superfamily multidrug transport protein
STM3125	-	1	Putative cytoplasmic protein
STM3789	<i>uhpB</i>	1	Sensor protein UhpB [EC=2.7.13.3]
STM4190	<i>pepE</i>	1	Peptidase E [EC=3.4.13.21]

Supplementary Table 2: Comparison of genotypic and phenotypic resistance or susceptibility for antimicrobials that can be used to treat invasive *Salmonella* infections. Phenotypic resistance was determined using Sensititre microbroth dilution. An isolate was classified genotypically resistant if it harboured one genotypic resistance marker or more. (*) For ciprofloxacin, decreased ciprofloxacin susceptibility was counted as "resistant". Genotypic data is listed in **Supplementary Data 1**. Phenotypic data is listed in **Supplementary Data 2**.

Antimicrobial	n° isolates tested	Phenotype susceptible		Phenotype resistant*		Concordance (%)
		Genotype resistant	Genotype susceptible	Genotype resistant	Genotype susceptible	
Ampicillin	56	0	22	34	0	100,00
Trimethoprim/Sulfamethoxazole	56	0	24	32	0	100,00
Chloramphenicol	56	0	22	33	1	98,21
Ceftriaxone	56	0	22	34	0	100,00
Ciprofloxacin	56	0	48	7	1	98,21
Azithromycin	56	0	50	6	0	100,00

Supplementary Table 3: Assembly summary of long read sequenced genomes.

The table is continued on the following page. Abbreviations: ONT = Oxford Nanopore Technologies, PacBio = Pacific Biosciences

Sequencing technology	Sequencing id	Strain	Contig type	Contig size (bp)	Depth of coverage	AMR genes
ONT	32640_1_296	0508H45184	Chromosome	4700258	157.15	no
			pSC_0508H45184 IncHI2A + IncA/C2	415479	403.47	yes
ONT	32640_1_316	1035531	Chromosome	4899417	631.26	yes
			pSC_1035531_1	100933	584.52	yes
			pSC_1035531_2 IncI1_Alpha	85904	1570.08	no
ONT	32640_1_328	H04340470	Chromosome	4751619	470.26	no
			pSC_H04340470	4028	1181.62	no
ONT	32640_1_336	H044240362	Chromosome	4660598	805.32	no
ONT	32640_1_364	70366	Chromosome	5009183	471.68	yes
			pSC_70366_1 IncA/C2	160357	483.10	yes
			pSC_70366_2	92366	276.92	no
ONT	32640_1_372	254833	Chromosome	4864180	45.82	yes
			pSC_254833_1	13181	349.20	yes
			pSC_254833_2 IncA/C2	71024	138.74	yes
			pSC_254833_3	100933	43.96	yes
			pSC_254833_4 IncI1_Alpha	85904	113.74	no
ONT	32640_1_39	64206	Chromosome	4800102	270.25	yes
			pSC_64206_1 IncHI2_1	372354	464.49	yes
			pSC_64206_2	106569	306.49	no
			pSC_64206_3	47794	438.69	no
			pSC_64206_4	5350	2375.96	no

Supplementary Table 3 continued

Sequencing technology	Sequencing id	Strain	Contig type	Contig size (bp)	Depth of coverage	AMR genes
ONT	32640_1_47	95907	Chromosome	4818922	186.13	yes
			pSC_95907_1 IncHI2A_1	288445	298.45	yes
			pSC_95907_2	106569	192.63	no
			pSC_95907_3	93718	136.40	no
			pSC_95907_4	47794	385.39	no
			pSC_95907_5	5350	735.74	no
ONT	SRS4345282	679052	Chromosome	4657242	296.78	no
ONT	SRS5777896	850890	Chromosome	4670214	138.10	no
			pSC_850890 IncHI2A	294257	171.69	yes
PacBio	Mapping reference ITM_8091960	1309	Chromosome	4811936	108.08	yes
			pSC_1309_1 IncHI2A	276676	191.66	yes
			pSC_1309_2	106569	193.88	no
			pSC_1309_3	93719	197.52	no
			uncircularised_contig	78477	163.25	no
			str1309_pSC_1309_4	5354	252.24	no

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