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1 **Global phylogeography and evolutionary history of *Shigella dysenteriae* type 1**

2

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115

116 **ABSTRACT**

117

118 Together with plague, small-pox and typhus, epidemics of dysentery have been a major
119 scourge of human populations for centuries¹. A previous genomic study concluded that
120 *Shigella dysenteriae* type 1 (Sd1), the epidemic dysentery bacillus, emerged and spread
121 worldwide after World War (WW) I, with no clear pattern of transmission². This is not
122 consistent with the massive cyclic dysentery epidemics reported in Europe during the
123 18th and 19th centuries^{1,3,4} and the first isolation of Sd1 in Japan in 1897⁵. We report here
124 a whole-genome analysis of 331 Sd1 isolates from around the world, collected between
125 1915 and 2011, providing us with unprecedented insight into the historical spread of this
126 pathogen. We show here that Sd1 has existed since at least the 18th century, and that it
127 swept the globe at the end of the 19th century, diversifying into distinct lineages
128 associated with WWI, WWII, and various conflicts or natural disasters across Africa,
129 Asia, and Central America. We also provide a unique historical perspective on the
130 evolution of antibiotic resistance over a 100-year period, beginning decades before the
131 antibiotic era, and identify a prevalent multiple antibiotic-resistant lineage in South Asia
132 that was transmitted in several waves to Africa, where it caused severe outbreaks of
133 disease.

134

135 **TEXT**

136

137 January 2016 marks one hundred years since the invasion force from Britain,
138 Australia, New Zealand and France withdrew from the Dardanelles, in the then Ottoman
139 Empire, only eight months after landing. Most of the more than 120,000 casualties
140 evacuated from the Gallipoli Peninsula were suffering from epidemic bacillary
141 dysentery⁶, caused by *Shigella dysenteriae* type 1^{7,8} (Sd1), a bacterium producing the
142 powerful Shiga toxin. This human-adapted clone of *Escherichia coli*⁹ was isolated for the
143 first time by Kiyoshi Shiga during a dysentery outbreak in Japan, during which 90,000
144 cases and 20,000 deaths occurred in the last six months of 1897 alone⁵. In the second half
145 of the 20th century, large outbreaks of disease due to Sd1 were still being reported in
146 Central America, with estimates of more than 500,000 cases and 20,000 deaths for the
147 1969-1973 epidemic^{10,11}, Africa, where there were an estimated 100,000 cases and 5-
148 10,000 deaths in the 1979 epidemic¹², and Asia^{13,14}.

149 Very little is known about the origins, evolution and spread of this important
150 human pathogen, including, in particular, the strains involved in the major outbreaks and
151 the genetic relationships between them. We carried out a whole-genome sequence
152 analysis on a set of Sd1 isolates selected from more than 35 international strain
153 collections, to represent the widest possible temporal and geographic distribution of
154 available isolates, to obtain a phylogenetic framework that was robust over time and
155 space and to infer transmission dynamics. This unique collection included 325 isolates
156 from 66 countries spanning four continents, collected between 1915 and 2011. Sixty-
157 seven historical isolates collected between 1915 and 1960, including 14 isolates obtained

158 during World War I (WWI)^{15,16}, were included in the collection, together with several
159 isolates from each major outbreak reported since the 1960s. Short-read sequences from
160 six Sd1 published genomes² were also included, with *S. flexneri*, *S. boydii*, *S. sonnei* and
161 *Escherichia coli* genomes used as outgroups.

162 Single-nucleotide polymorphisms (SNPs) were detected by mapping short-read
163 sequences against Sd1 reference genomes: Sd197¹⁷, which was isolated during an
164 outbreak in China in the 1950s, and Sd1617¹⁸, which was isolated in Guatemala during
165 the 1968-1969 epidemic. Maximum likelihood (ML) phylogenetic analysis was
166 performed on 14,677 (mapping against Sd197) and 15,752 (mapping against Sd1617)
167 chromosomal SNPs, which were randomly distributed over the non-repetitive non-
168 recombinant core genome (85.6% of the Sd197 chromosome, Supplementary
169 Information). Four genetic lineages (Fig. 1a, Supplementary Information) were identified.
170 Lineage I contained only M115, which was isolated from a case in England in 1926.
171 Lineage II contained mostly isolates collected in Europe between 1915 and 1958.
172 Lineage III contained isolates from around the world and could be split into four
173 sublineages with strong geographical affinities: IIIa in eastern and southeastern Asia
174 (with isolates collected between 1927 and 1971), IIIb in Central America (1955-1992),
175 IIIc in West Africa (1954-2006), and IIId in southern Asia and eastern Africa (1956-
176 1977) and then in West Africa (1979-1998). Finally, lineage IV contained most of the
177 Sd1 isolates obtained from the Indian subcontinent and Africa in the last few decades.

178 Ten of the 14 isolates (71%) amassed by Captain E.G.D. Murray during WWI
179 belonged to the European lineage, lineage II, and most were isolated at the 2nd Western
180 General Hospital, Manchester, which received many of the soldiers evacuated during the

181 Gallipoli campaign (Supplementary Fig. 1). The other four isolates belonged to three of
182 the four sublineages of the global lineage, lineage III. None of the WWI isolates belonged
183 to sublineage IIIId, which gave rise to the modern lineage, lineage IV.

184 The two candidate vaccine strains developed to date are derived from lineage III
185 parental isolates (IIIb for parental strain Sd1617 of vaccine strain WRSd1¹⁹ and IIIId for
186 parental strain 7-87 of vaccine strain SC-599²⁰).

187
188 ML phylogenetic analysis revealed a strong correlation between root-to-tip branch
189 lengths and the known years of isolation for the sequenced Sd1 isolates, indicating a
190 clock-like evolution (Supplementary Fig. 2). We therefore used a Bayesian phylogenetic
191 approach to provide estimates of the nucleotide substitution rates and divergence times of
192 the different lineages for a spatially and temporally representative subset of 125 isolates
193 (Fig. 2). We estimated the genome-wide substitution rate at 8.7×10^{-7} substitutions site⁻¹
194 year⁻¹ [95% credible interval (CI) = $7.6 \times 10^{-7} - 9.9 \times 10^{-7}$], giving a most recent common
195 ancestor (MRCA) for all the Sd1 in our collection dating from 1747 (95% CI, 1645 -
196 1822). This finding is consistent with historical data from the 18th to mid-19th centuries,
197 describing cyclic dysentery epidemics in Western and Northern Europe associated with
198 extraordinarily high mortality rates. For example, the 1738-1742 and 1779 epidemics in
199 France killed more than 200,000 people¹, the 1770-1775 epidemic in Sweden killed
200 almost 35,000 people (12% of all deaths during the period)³, and a large number of
201 deaths from dysentery were also reported during the Irish Great Famine of 1846-1849⁴.
202 The MRCA for all isolates other than M115 was dated to the mid-19th century (1853;
203 95% CI 1831-1871), whereas the MRCAs for each of the sublineages of global lineage

204 III were estimated to have existed between 1889 (95% CI 1881-1897) and 1903 (95% CI
205 1893-1913), indicating that this lineage spread worldwide over a period of less than two
206 decades. This dating is also consistent with Shiga's observation that the dysentery
207 outbreak of 1897 had begun in the late 1880s in the southern part of Japan²¹.

208 Our findings show that the global spread of Sd1 predates WWI. It therefore
209 occurred earlier than for another *Shigella* serogroup, *S. sonnei*, which has been shown to
210 have spread to other continents from Europe during the second half of the 20th century²².
211 We cannot demonstrate causality between the spread of Sd1 and historical events on the
212 basis of the results presented here, but the late 1800s coincided with a period of intense
213 European emigration, the colonisation of various territories in Africa and Asia by
214 European powers, facilitated by the opening of the Suez canal (1869) and the
215 development of steamships.

216

217 Geographic and temporal analyses identified several intercontinental transmission
218 events resulting in long-term establishment of the bacterium (Figs 1b, 1c, and 2).
219 Transmission event T1 involved the European lineage II and led to an introduction of Sd1
220 in Madagascar between 1915 (95% CI 1910-1921) and 1967 (95% CI 1956-1977), during
221 French colonization. This is consistent with the first report, which unambiguously
222 described Sd1 there in 1927²³. Transmission event T2, involving eastern Asia and Poland,
223 is estimated to have occurred between 1910 (95% CI 1899-1925) and 1944 (95% CI
224 1942-1945). All other transmission waves originated in the Indian subcontinent and
225 affected mostly East Africa. Two of these transmission waves, T5 and T8, led to major
226 outbreaks; according to our estimates, T5 occurred between 1970 (95% CI 1963-1975)

227 and 1979 (95% CI 1976-1981). This dating is consistent with the first reported outbreak
228 in the northeastern part of what is now the Democratic Republic of the Congo in 1979, 28
229 years after the last isolation of Sd1 in Central Africa¹². This epidemic then spread to the
230 Great Lakes region, where it persisted until at least 1990¹². T8 occurred between 1984
231 (95% CI 1978-1987) and 1987 (95% CI 1985-1989), with a first reported outbreak in
232 Zambia in 1990-1991^{12,24}. The strain then rapidly spread across an Africa ravaged by
233 civil unrest, war (e.g., Mozambique, Angola, Rwanda, Sierra Leone) and HIV
234 infection^{12,24} until 2011. With the exception of a localized outbreak in the northern part of
235 the Central African Republic in 2004²⁵ caused by sublineage IIIc (see below), all other
236 outbreaks in Africa since 1990 have been caused by lineage IV.

237

238 The high resolution of whole-genome sequence analysis (WGS) has significantly
239 changed our understanding of the patterns of Sd1 transmission over time at a global scale.
240 The classical molecular epidemiology tools (Supplementary Information) previously used
241 were unable to unravel these patterns of transmission. Furthermore, a re-evaluation of
242 two outbreaks that occurred in the Central African Republic in 2003-2004²⁵ that we had
243 previously investigated by pulsed-field gel electrophoresis (PFGE), the current method of
244 choice for subtyping Sd1, revealed a lack of correlation between PFGE and WGS data
245 (Supplementary Fig. 3 and Supplementary Information). In particular, PFGE grouped the
246 isolates from the two outbreaks closely together, whereas they actually belonged to two
247 different lineages, IIIc and IV, separated by ~700 SNPs. By contrast, other African T8
248 lineage IV isolates differing by 37 to 61 SNPs from the Central African Republic T8
249 lineage IV outbreak isolates, formed a more distant group. Thus, PFGE cannot attribute

250 profiles from different apparently geographically restricted outbreaks to a single, longer
251 epidemic, such as that associated with the T8 transmission wave in Africa. PFGE should,
252 therefore, no longer be used for the assessment of phylogenetic relationships in Sd1.
253 Instead, WGS provides a robust phylogenetic framework for the epidemiological tracking
254 of this bacterium.

255

256 One key feature in the evolution of Sd1 is the acquisition and accumulation of
257 antibiotic resistance genes (ARGs) (Figs 3, 4, Supplementary Fig. 4, and Supplementary
258 Information). The first antibiotic-resistant Sd1 isolates in our collection were recovered in
259 Asia and America during the 1960s and rapidly became predominant, such that
260 susceptible isolates had become exceptional by 1991 (100%, [67/67] susceptible isolates,
261 between 1915 and 1960 and <1% [1/123], between 1991 and 2011). Lineage IV, the most
262 recent of the lineages identified, is the most affected by antibiotic resistance, but almost
263 all the contemporary circulating strains from older lineages have also become resistant to
264 multiple antibiotics. ARGs were acquired following the first use of antibiotics in clinical
265 practice (Fig. 4b). The first ARGs identified in Sd1 were borne on small plasmids (<10
266 kb), encoding resistance to streptomycin and sulfonamides. Larger plasmids (80-130 kb)
267 of different types encoding additional resistance to tetracycline, chloramphenicol, and,
268 for some plasmids, ampicillin (via the *bla*_{OXA-1} or *bla*_{TEM-1} genes) were then acquired in
269 various geographic areas, from the mid-1960s to the 1980s. These plasmids belonged to
270 the IncK and IncF groups in Asia and to the IncB/O group in Central America. The use of
271 cotrimoxazole, beginning in the late 1960s, led to the acquisition of dihydrofolate
272 reductase genes, mostly *dfrA1*, carried by 110-kb pST186 IncI1 and 30-kb IncX4

273 plasmids or by the Tn7 transposon inserted into the Sd1 chromosome close to the *glmS*
274 gene, as observed for *S. sonnei*²². Since the 1990s, the principal structure associated with
275 multidrug resistance in Sd1 has been a 66-kb genomic element called the *Shigella*
276 resistance locus pathogenicity island (SRL-PAI)²⁶. It was acquired four times in lineage
277 IV (South Asia or the Middle East), once in sublineage IIIc (West Africa), and once in
278 lineage II (Madagascar). Further evidence for the independent acquisition of the SRL-
279 PAI is provided by the presence of slight differences between the different acquired SRL-
280 PAIs (Supplementary Fig. 5). The SRL-A is very similar to the first SRL-PAI to be
281 described in *S. flexneri*²⁶ and it was found exclusively in lineage IV. The SRL-B, found
282 only in the lineage IV African T8 isolates, was probably derived from the SRL-A by
283 insertion sequence (IS) *ISSdI*-mediated rearrangements rather than being independently
284 acquired. The other SRL-PAI contained various insertions (group II introns, part of the
285 *shf* operon, region replacing *orf47*) not present in SRL-A. Among the 149 isolates
286 bearing the SRL-PAI, only two showed a partial deletion of the SRL-PAI, resulting in a
287 loss of the antibiotic resistance cluster (i.e., the SRL *sensu stricto*). This structure is
288 therefore quite stable over time, particularly in a bacterium containing hundreds of
289 ISs^{17,18}. This 66-kb element encodes resistance to ampicillin, streptomycin,
290 chloramphenicol and tetracycline, with no more resistance than the previously circulating
291 large plasmids. Its persistence may therefore be associated with a lower fitness cost and
292 the presence of an *fec* operon for the capture of iron, serving as selective advantages²⁶.
293 Before the principal acquisition of the SRL-A, the closest ancestral group (consisting
294 initially of South Asian and then South-East and Central Asian isolates), had acquired a
295 chromosomally encoded transposon (Fig. 2, Supplementary Fig. 6). This 10-kb structure

296 encodes resistance to chloramphenicol and tetracycline. The structure of the double drug-
297 resistance module is similar to that found in the SRL and to some previously circulating
298 large multidrug resistance IncF plasmids, such as p3099-85 and p80-547. This recent
299 trend towards acquiring ARG-containing genomic islands or chromosomally-encoded
300 transposons rather than plasmids is also displayed by the 7th pandemic *V. cholerae*
301 (SXT/R391) and *Salmonella enterica* serotype Typhi H58 (24-kb composite transposon)
302 strains, which also originate from the Indian subcontinent^{27,28}.

303 Resistance to nalidixic acid, a quinolone, mediated by point mutations in the DNA
304 gyrase gene, *gyrA*, was acquired seven times in lineage IV Sd1 isolates from South Asia
305 and Africa (Fig. 2) from the 1980s. The *gyrA* mutation leading to a serine-to-leucine
306 substitution in the amino-acid sequence, S83L was the most frequently observed, but
307 others, involving codon 87, such as D87G and D87Y, were observed in isolates from
308 Central Africa and Thailand, respectively, during the 1990s. Interestingly, in the same
309 geographic area of DRC and Rwanda in 1994, two different mutations were acquired
310 (S83L and D87G). This may reflect the heavy use of nalidixic acid in the Rwandan
311 refugee camps, which experienced outbreaks of disease caused by *Vibrio cholerae* O1
312 and Sd1²⁹.

313 Resistance to ciprofloxacin, a fluoroquinolone, mediated by a double mutation in
314 *gyrA* (S83L and a second mutation in codon 87) and a mutation in the topoisomerase IV
315 *parC* gene (S80I) was acquired only once, in a group of 20 isolates from the Indian
316 subcontinent collected between 1995 and 2010 (Fig. 2). We observed no resistance to
317 extended-spectrum cephalosporins, carbapenems or azithromycin in the isolates studied
318 here, but the existence of such resistance is almost inevitable, as the area of circulation of

319 Sd1 overlaps with that of Enterobacteriaceae possessing mobile ARGs encoding
320 resistance to the latest generation of antibiotics, such as NDM-1³⁰. However, the dramatic
321 decrease in Sd1 isolation reported since the turn of the century and not explained by the
322 findings of this genomic study, may counterbalance these pessimistic predictions.

323

324 **METHODS**

325

326 **Bacterial isolates**

327

328 The Sd1 isolates analysed in this study are listed in Supplementary Table 1 and originated
329 from the collections of the Centers for Disease Control and Prevention, Atlanta, GA,
330 USA (*n*=56); Institut Pasteur, Paris, France (*n*=53); Public Health England, Colindale,
331 UK (*n*=29); Iccdr,b, Dhaka, Bangladesh (*n*=29); Central Research Institute for
332 Epidemiology, Moscow, Russian Federation (*n*=22); National Institute of Public Health,
333 Prague, Czech Republic (*n*=19); Public Health England, Porton Down, UK (*n*=17); Iris-
334 Lab, Brussels, Belgium (*n*=11); National Institute of Cholera and Enteric Diseases,
335 Kolkata, India (*n*=8); Institut Pasteur de Bangui, Bangui, Central African Republic (*n*=7);
336 Norwegian Institute of Public Health, Oslo, Norway (*n*=6); Hungarian National
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342 ($n=3$); Bégin Military Hospital, Saint-Mandé, France ($n=3$); IAME, Paris, France ($n=3$);
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349 ($n=1$); Istituto Pasteur-Fondazione Cenci Bolognetti, Rome, Italy ($n=1$); Félix d'Hérelle
350 reference center for bacterial viruses, Université Laval, Québec, Canada ($n=1$); National
351 Institute for Communicable Disease Control and Prevention, Beijing, China ($n=1$).

352

353 Bacterial DNA samples were also received from the Armed Forces Research Institute of
354 Medical Sciences, Bangkok, Thailand ($n=10$).

355

356 The 18 Sd1 isolates from the E.G.D. Murray collection^{15,16,31} included 14 isolates
357 recovered during WWI and four isolates obtained between 1926 and 1930. The WWI
358 isolates were obtained from different sources (Supplementary Fig. 1) and were stored at
359 room temperature in Douglas digest agar slant glass tubes after sealing with a gas-air
360 burner between August 1918 and October 1919. In 1980, the 18 tubes and the 680 other
361 cultures of Enterobacteriaceae from the entire collection were shipped to the National
362 Collection of Type Cultures (NCTC), Porton Down, UK, opened and freeze-dried.

363

364 It was confirmed that all the isolates included belonged to Sd1, by conventional methods
365 and serotyping at the French National Reference Center for *E. coli*, *Shigella* and
366 *Salmonella*, Institut Pasteur, Paris, as previously described³².

367

368 **Antibiotic susceptibility testing**

369

370 Antibiotic susceptibility was determined by disk diffusion on Mueller-Hinton (MH) agar
371 in accordance with the guidelines of the Antibiogram Committee of the French Society
372 for Microbiology (CA-SFM 2014) (www.sfm-microbiologie.org/). The following
373 antimicrobial drugs (Bio-Rad, Marnes-la-Coquette, France) were tested: amoxicillin,
374 ceftriaxone, ceftazidime, streptomycin, kanamycin, amikacin, gentamicin, nalidixic acid,
375 ofloxacin, ciprofloxacin, sulfonamides, trimethoprim, sulfamethoxazole-trimethoprim,
376 chloramphenicol, tetracycline, and azithromycin. *Escherichia coli* CIP 76.24 (ATCC
377 25922) was used as a control. For strains displaying resistance to either nalidixic acid or
378 ciprofloxacin by the disk diffusion method, this resistance was confirmed by
379 determination of the minimal inhibitory concentration (MIC) with the corresponding
380 Etest strips (bioMérieux, Marcy L'Etoile, France). The MICs of azithromycin and
381 nitrofurantoin were determined by Etests for 30 isolates chosen on the basis of resistance
382 phenotype, and year and country of isolation.

383

384 **Determination of the mutator phenotype of strain M115**

385

386 The mutation rate of M115 was estimated by monitoring the capacity of this strain to
387 generate mutations conferring resistance to rifampin in two independent experiments
388 including duplicates, as previously described³³. *E. coli* strain ECOR48 (CIP 106023) was
389 used as a strong mutator positive control³⁴, the Sd1 97-13397 isolate was used as a
390 putative strong mutator isolate (deletion of the *mutS* gene), Sd1 M116 and Sd197 were
391 used as putative normomutator isolates (integrity of the *mutS*, *mutH*, *mutL* and *uvrD*
392 methyl-directed mismatch repair genes).

393

394 **Total DNA extraction**

395

396 Total DNA was extracted with the InstaGene matrix kit (Bio-Rad) for the PCR
397 identification of antibiotic resistance genes, the Wizard Genomic DNA Kit (Promega,
398 Madison, WI, USA) for multilocus sequence typing and Illumina sequencing and the
399 phenol chloroform method³⁵ for Illumina sequencing and PacBio sequencing.

400

401 **Multi-locus sequence typing**

402

403 Conventional multi-locus sequence typing (MLST) was performed on a subset of 33 Sd1
404 isolates, as previously described³⁶. Sequencing was performed at the *Plateforme de*
405 *Génotypage des Pathogènes et Santé Publique*, PF8 (Institut Pasteur). The nucleotide
406 sequences and deduced protein sequences were analysed with EditSeq and Megalign
407 software (DNASTAR, Madison, WI, USA). The BLASTN program of NCBI was used
408 for database searches (<http://www.ncbi.nlm.nih.gov/BLAST/>).

409

410 **PCR identification of antibiotic resistance genes**

411

412 The *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA-1}, *cat1*, *sul1*, *dfrA1*, and *aadA1* resistance genes and the class
413 1 and 2 integron gene cassettes were amplified by PCR, as previously described³⁷.

414

415 The presence of the *Shigella* resistance locus pathogenicity island (SRL-PAI) was
416 assessed by PCR, as previously described³⁸. The structure of the SRL-PAI was assessed
417 by PCR mapping with the primers described or with new primers designed on the basis of
418 GenBank accession no. AF326777. Amplicons not of the expected size were sequenced.

419

420 **Plasmid analyses**

421

422 Plasmids were obtained from *E. coli* transconjugants or transformants, as previously
423 described³⁷, except that ampicillin (50 mg/L) or chloramphenicol (20 mg/L) was used as
424 a selective agent.

425

426 Plasmid size was determined in parental and transconjugant or transformants strains by
427 S1 nuclease treatment and pulsed-field gel electrophoresis, as previously described³⁷.

428 PCR-based replicon-typing analysis was performed as previously described³⁹.

429

430 Eight 30-130 kb plasmids conferring antimicrobial resistance were sequenced. Plasmid
431 DNA was extracted with the Large-Construct Kit (Qiagen, Courtaboeuf, France) and

432 sequenced through services provided by GATC Biotech (Konstanz, Germany), using
433 shotgun sequencing runs on a 454/Roche GS FLX Analyzer (Roche, Basel, Switzerland).
434 The resulting sequences were assembled into a unique scaffold. Gap closure was carried
435 out by PCR followed by Sanger DNA sequencing with the Big Dye® Terminator V3.1
436 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and a 96-capillary
437 3730xl DNA Analyzer (Applied Biosystems), by Eurofins MGW Operon (Cochin
438 Platform, Paris, France). Automatic annotation was performed with the RAST⁴⁰
439 server (<http://rast.nmpdr.org/>), followed by manual inspection and correction. The
440 sequences obtained have been deposited in GenBank under the accession numbers
441 KT754160 (p80-547), KT754161 (pCAR10), KT754162 (pBU53M1), KT754163
442 (pA5468), KT754164 (p3099-85), KT754165 (p93-531-1), KT754166 (p92-9000),
443 KT754167 (p69-3818).

444

445 **Whole-genome sequencing**

446

447 High-throughput genome sequencing was carried out at the genomics platform of the
448 Pasteur Institute, GATC Biotech, Beckman Coulter Genomics (Danvers, MA, USA) or at
449 the Wellcome Trust Sanger Institute, on Illumina platforms generating 100 to 146 bp
450 paired-end reads, yielding a mean of 206-fold coverage (minimum 37-fold, maximum
451 990-fold) (Supplementary Table 2). Short-read sequence data were submitted to the
452 European Nucleotide Archive (ENA) (<http://www.ebi.ac.uk/ena>) and the genome
453 accession numbers are provided in Supplementary Table 1.

454

455 We optimised the resolution of the chromosome-encoded antibiotic resistance structures
456 and ensured that representative isolates from the various lineages were included, by
457 sequencing 10 isolates on the PacBIO RS II platform (Pacific Biosciences, CA, USA), as
458 previously described²⁸. The PacBio data were submitted to the ENA and the genome
459 accession numbers are provided in Supplementary Table 1.

460

461 **Other studied genomes**

462

463 Sd1 strain Sd197¹⁷ was used as the reference genome. A second Sd1 genome Sd1617¹⁸
464 was used as a second reference genome, to confirm the population structure found with
465 Sd197.

466

467 Short-read sequences from the following six Sd1 genomes published by Rohmer *et al.*²
468 were downloaded from the ENA and included in this study: 2735 (USA, 1974,
469 SRR765065), 91R17 (Guatemala, 1991, SRR765098), 91R14 (Guatemala, 1991,
470 SRR765104), DH03 (Central African Republic, 1996, SRR765110), DH05 (Central
471 African Republic, 1996, SRR765112), and DH06 (Central African Republic, 1996,
472 SRR765113).

473

474 The following genomes were used as outgroups: *E. coli* O157:H7 strain Sakai (GenBank
475 accession no. NC_002695), *E. coli* strain K-12 MG1655 (GenBank accession no.
476 NC_000913), *S. flexneri* type 2a strain 2457T (GenBank accession no. AE014073), *S.*

477 *boydii* strain Sb227 (GenBank accession no. NC_007613), and *S. sonnei* strain Ss046
478 (GenBank accession no. NC_007384).

479

480 **Read alignment and SNP detection**

481

482 For the analysis of single-nucleotide polymorphisms (SNPs), Illumina-generated paired-
483 end reads and the simulated paired-end reads from publicly available assembled
484 genomes, were mapped to the reference genome of Sd1 strain Sd197, including the
485 chromosome (CP000034) and plasmids pSD1_197 (CP000035) and pSD197_spA
486 (CP000640), with SMALT (version 0.7.4)
487 (<http://www.sanger.ac.uk/resources/software/smalt/> as previously described²⁸.

488

489 ***De novo* assembly**

490

491 The reads for each strain were assembled *de novo* with Velvet⁴¹ version 1.2.09, with
492 parameters optimised with VelvetOptimiser version 2.2.5
493 (<https://github.com/tseemann/VelvetOptimiser>). They were scaffolded with SSPACE⁴²
494 version v2.0. The gaps were closed with GapFiller⁴³ version 1.11, and the sequences were
495 annotated with Prokka⁴⁴ version 1.5, as previously described²⁸. CLC Assembly Cell
496 version 4.2.0 (CLC bio, Aarhus, Denmark) was also used to investigate antibiotic
497 resistance determinants.

498

499 **Phylogenetic analyses**

500

501 The maximum likelihood (ML) phylogenetic tree shown in Supplementary Fig. 7 was
502 built from a 140,385-chromosomal SNP alignment generated by `snp_sites` software
503 (https://github.com/sanger-pathogens/snp_sites) from all 331 short-read sequences, plus
504 Sd1 genomes Sd197 (used as a reference) and Sd1617, together with the six *E. coli* and
505 *Shigella* sp. genomes used as outgroups. RAxML⁴⁵ version 7.8.6 was used with the
506 generalised time-reversible model and a Gamma distribution to model site-specific rate
507 variation (the GTR+ Γ substitution model; GTRGAMMA in RAxML). Support for the
508 ML phylogeny was assessed by 100 bootstrap pseudo-analyses of the alignment data, and
509 the final tree was visualised in FigTree version 1.4.2
510 (<http://tree.bio.ed.ac.uk/software/figtree/>).

511

512 The ML phylogenetic trees shown in Figs 1a, 3a, 3c, Supplementary Figs 1a, 3b, 4, 9, 11
513 and 14 were built from a 14,677-chromosomal SNP alignment of all 331 Sd1 short-read
514 sequences, plus Sd1 genome Sd197, used as the reference. Repetitive regions (within the
515 chromosome, between the chromosome and the virulence plasmid (VP) or the SRL-PAI)
516 were removed manually with the Artemis⁴⁶ genome browser. Recombinogenic regions
517 were also removed with the Gubbins⁴⁷ software. The remaining 14,677 chromosomal
518 SNPs were randomly distributed along the non-repetitive non-recombinant core genome
519 (3,750,125 bp), with a spacing of about one SNP per 256 bp or a nucleotide divergence of
520 0.39% (Supplementary Fig. 12). RAxML version 7.8.6 (GTRGAMMA substitution
521 model) was used to construct the tree. We performed 500 bootstrap pseudoreplicate
522 analyses to assess support for the ML phylogeny. The tree was rooted on M115, which

523 was shown to be the most closely related to the ancestral strain of Sd1 by two different
524 approaches (ML and Bayesian) and was visualised with MEGA⁴⁸ version 6, iTOL^{49,50} or
525 FigTree version 1.4.2.

526

527 The ML phylogenetic trees shown in Supplementary Figs 10 and 11 were built from a
528 15,752-chromosomal SNP alignment of all 331 Sd1 short-read sequences, plus Sd1
529 genome Sd1617, used as the reference. The method used was similar to that described
530 above, except that the repetitive regions were not removed manually and phylogenetic
531 support was assessed by 100 bootstrap pseudo-analyses.

532

533 The VP phylogenetic tree shown in Supplementary Fig. 15 was constructed similarly,
534 from the 226 plasmid-containing isolates (> 90% coverage at read depth > 10x), based on
535 290 SNPs randomly distributed along the non-repetitive non-recombinant pSD1_197
536 sequence (99,704 bp, 54.6% of pSD1_197). The tree was unrooted.

537

538 **Phylogenetic clustering**

539

540 We clustered the isolates of Sd1 into various lineages by eye and by applying hierarchical
541 Bayesian analysis of population structure (BAPS)⁵¹ software to the 14,677-chromosomal
542 SNP alignment. Five iterations (L value) were run with a maximum cluster number (K
543 value) of 6 or 10 and three iterations were run with $K=6$.

544

545 **Temporal analysis**

546

547 We investigated the temporal signal in the ML phylogeny for Sd1, using Path-O-Gen
548 (<http://tree.bio.ed.ac.uk/software/pathogen/>). The relationships between root-to-tip
549 distances, year of isolation and lineage were analysed by linear regression methods.

550

551 We used Bayesian Evolutionary Analysis by Sampling Trees (BEAST)⁵² version 1.8 to
552 date the important nodes. The analyses were conducted on a subsample of 125 isolates
553 from across the ML tree, covering the full temporal and geographic range of this
554 pathogen. The concatenated 10,798 chromosomal SNP alignments of these 125 strains
555 were subjected to multiple BEAST analyses with both constant-size and Bayesian skyline
556 population size change models, in combination with either a strict molecular clock or a
557 relaxed clock, to identify the best-fit model^{22,53}. For the BEAST analysis, the GTR+ Γ
558 substitution model was selected and tip dates were defined as the year of isolation. For all
559 model combinations, three independent chains of 100 million generations each were run
560 to ensure convergence, with sampling every 1,000 iterations. Convergence and effective
561 sample size (ESS) values were inspected using Tracer⁵² version 1.5. A marginal
562 likelihood estimation was carried out, with path sampling and stepping stone sampling
563 for each run that had converged, to compare the different combinations of clock and tree
564 models^{54,55}. The marginal likelihood estimation was then used to determine which model
565 gave the best fit, by calculating the Bayes Factor. The relaxed, uncorrelated lognormal
566 clock model, which allows evolutionary rates to vary among the branches of the tree
567 together with the skyline demographic model proved a much better fit for the data, as
568 found previously for *S. sonnei*²² and *S. flexneri*⁵³. The parameter and tree estimates of the

569 three runs were combined with LogCombiner⁵² version 1.7.5, with the first 20% of states
570 in each chain removed as burn-in. Maximum clade credibility (MCC) trees were
571 generated with TreeAnnotator⁵² version 1.7.5 on the combined files, and visualised with
572 FigTree version 1.4.2. Estimates are reported as median values with the 95% highest
573 posterior density (HPD, hereafter referred to as the credible interval). The Bayesian
574 skyline plot was calculated and visualised with Tracer⁵² version 1.5, to investigate
575 changes in the effective population size of Sd1 over time. To confirm the dating
576 estimates, ten other random subsamples were generated from clusters calculated using the
577 Prosperi method⁵⁶ (code here:
578 http://figshare.com/articles/clustertree.R_Code_for_clustering_phylogenetic_trees/97225)
579 with a threshold of 0.03. All singleton isolates were included (n=86) and one isolate from
580 each of the 33 clusters was randomly selected to generate the ten subsamples. These
581 alignments were analysed in BEAST using the same model and showed similar dating for
582 each of the lineages (Supplementary Table 2).

583

584 **Genetic analyses**

585

586 *In silico* MLST was then carried out by MLST version 1.8
587 (<https://cge.cbs.dtu.dk/services/MLST/>) on assembled sequences for all the dataset. New
588 alleles were confirmed by Sanger sequencing and submitted to the MLST database
589 website (<http://mlst.warwick.ac.uk/mlst/>).

590

591 The presence and type of antibiotic resistance genes (ARGs) or ARG-containing
592 structures (Fig. 3b and Supplementary Fig. 4) were determined with ResFinder⁵⁷ version
593 2.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>), BLAST analysis against defined
594 reference sequences (plasmids or chromosomally encoded structures), PlasmidFinder⁵⁸
595 version 1.3 (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>), Plasmid MLST
596 locus/sequence definitions database (<http://pubmlst.org/plasmid/>), and pMLST version
597 1.2 (<https://cge.cbs.dtu.dk/services/pMLST/>) on CLC or Velvet assemblies. The new
598 alleles and STs of IncI⁵⁹ and IncN⁶⁰ plasmids have been deposited in the PubMLST
599 database (<http://pubmlst.org/plasmid/>). The presence of mutations in the quinolone-
600 resistance determining region of the DNA gyrase and topoisomerase IV genes was
601 determined manually on *de novo* assembled sequences. PacBio sequences were used to
602 analyse the structure of the SRL-PAI variants and the composite transposon inserted into
603 the chromosome in genome CDC 87-3330. The *in silico* results were compared with PCR
604 data, when available.

605

606 **Pan-genome analysis**

607

608 Roary⁶¹ version 3.2.4 was used on Velvet-annotated assemblies, to construct a pan-
609 genome. The pan-genome analysis identified genome 2735² as an outlier. Further
610 investigation revealed an extreme AT bias, therefore this sample was excluded from
611 subsequent analyses. A more sensitive annotation was performed on the resulting clusters
612 of proteins with InterPro⁶², to provide Gene Ontology⁶³ classifications for each gene.

613

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773 **SUPPLEMENTARY INFORMATION**

774 Supplementary Information is linked to the online version of the paper at

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776

777 The authors declare no competing financial interests.

778

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781

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803

804 **AUTHOR CONTRIBUTIONS**

805

806 R.B., P.A.D.G., S.B., N.R.T and F.-X.W. designed the study. N.S., C.J., K.A.T., R.B.,
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813 extractions. A.E.M. and S.R.H provided guidance for genomic analyses. C. Bouchier
814 performed the whole-genome sequencing. M.A. processed the short reads. E.N.-N., N.F.,
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816 data. F.-X.W. wrote the manuscript with major contributions from A.E.M., P.A.D.G.,
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818 F.-X.W. oversaw the project.

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820 **AUTHOR INFORMATION**

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822 Short-read sequences have been deposited at EBI-ENA, under study accession numbers
823 PRJEB10304, PRJEB2846 and PRJEB3255. PacBio sequences have been deposited at
824 EBI-ENA, under study accession number PRJEB7928. Plasmid, SRL-PAI, and Tn87-
825 3330 sequences have been deposited in GenBank, under accession numbers KT754160–
826 KT754167, KT777637–KT777641, and KT777642, respectively.

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830 **TABLE**

831 None

832

833 **FIGURE LEGENDS**

834

835 **Figure 1. Geographic distribution and transmission patterns of *Shigella dysenteriae***
836 **type 1 genetic lineages. a**, Maximum likelihood (ML) phylogeny of the 332 genomes
837 studied, showing the four lineages, I to IV, and the four sublineages of lineage III: IIIa to
838 IIIId. The tree was rooted on M115, the most closely related to the *S. dysenteriae* type 1

839 ancestral strain. The tips of the tree are coloured to indicate the continent on which the
840 infection occurred. T1 to T8 indicate intercontinental transmission events. **b**, Geographic
841 presence (circles), inferred arrivals (thick arrows) and principal long-distance
842 transmission events (thin arrows) of lineages I to III based on phylogeographic analysis.
843 Intercontinental transmission events are indicated by the letter T. The date ranges shown
844 for transmission events are the median values for the MRCA (taken from BEAST) with
845 the first number indicating the median MRCA of the transmitted strains, and the second
846 number indicating the median MRCA of the transmitted strains and their closest relative
847 from the source location. **c**, Geographic presence (circles, thunderbolts) and
848 intercontinental transmission events of lineage IV based on phylogeographic analysis.
849 Isolate assignment to the corresponding transmission event is indicated by coloured
850 halos.

851

852 **Figure 2. Timed phylogeny of a subsample of 125 *Shigella dysenteriae* type 1 isolates.**

853 **a**, Bayesian skyline plot showing temporal changes since 1747 in effective population
854 size (black curve) with 95% confidence intervals (cyan). World War I (WWI) is indicated
855 by a red bar. **b**, Maximum clade credibility tree produced using BEAST (lognormal
856 relaxed clock model; Bayesian skyline) also presenting information about the ortho-
857 nitrophenyl- β -galactoside (ONPG) test. Resistance to nalidixic acid (NAL^R) is indicated
858 by a purple circle and resistance to ciprofloxacin (CIP^R) is indicated by a purple triangle.
859 Acquisition of the antibiotic resistance element, *Shigella* resistance locus pathogenicity
860 island (SRL-PAI), is indicated by a black thunderbolt. Acquisition of the resistance
861 transposon (Tn87-3330), originally found in isolate CDC 87-3330, is indicated by an

862 orange thunderbolt. T1 to T8 indicate intercontinental transmission events. Estimated
863 dates for the intercontinental transmission events are provided in dataset S7 of
864 Supplementary Table 2.

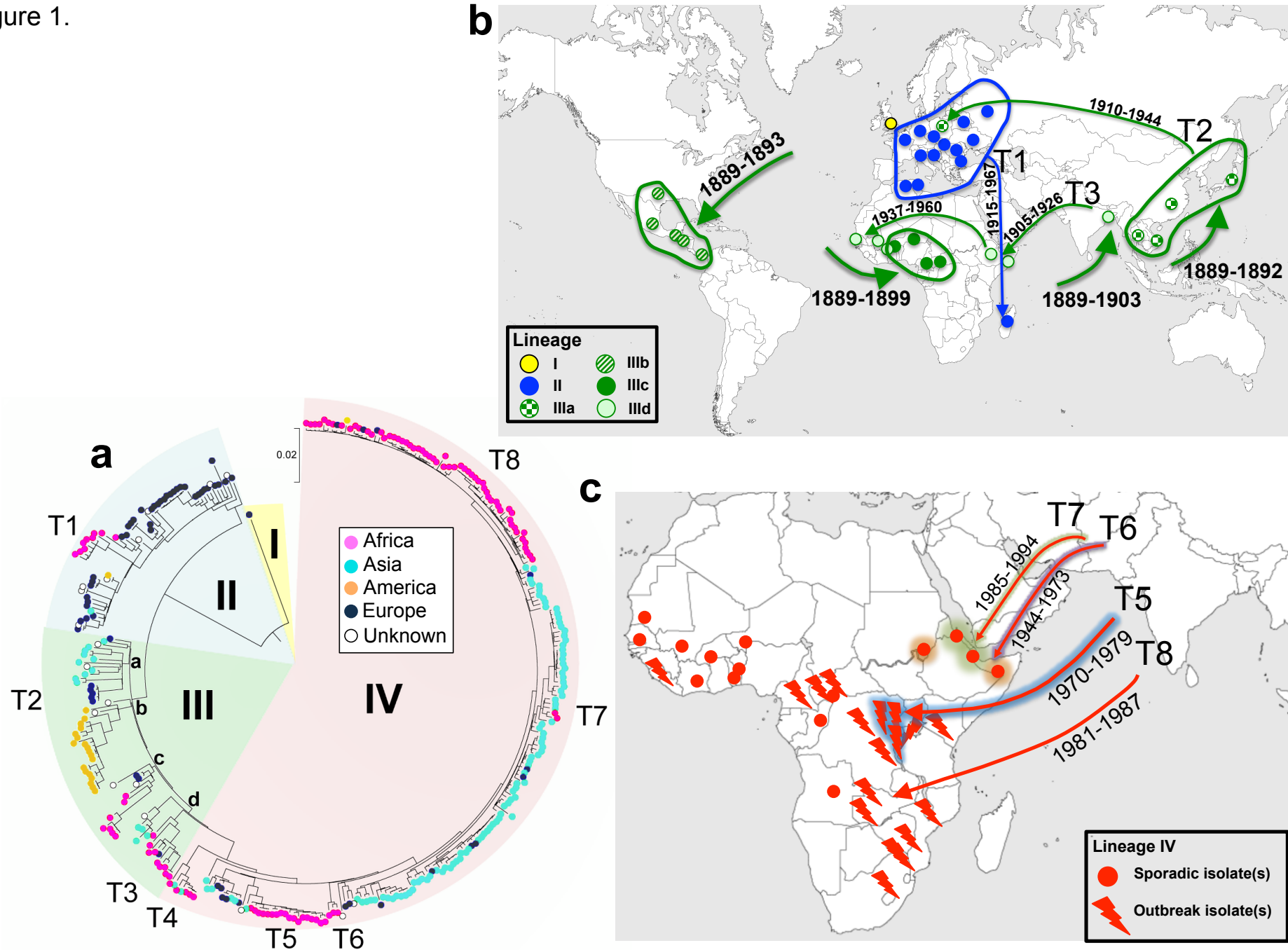
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866 **Figure 3. Phenotypic and genetic characterization of antibiotic resistance in *Shigella***
867 ***dysenteriae* type 1. a**, Resistance phenotype for eight antibiotics (ampicillin, AMP;
868 streptomycin, STR; sulfonamides, SUL; trimethoprim, TMP; chloramphenicol, CHL;
869 tetracycline, TET; nalidixic acid, NAL; and ciprofloxacin, CIP), according to the lineages
870 (I to IV) defined on the basis of the maximum likelihood (ML) phylogeny (as in Fig. 1a).
871 Resistance is indicated in red and susceptibility in grey, whereas no antibiotic
872 susceptibility data is indicated in white. **b**, Principal genetic structures bearing antibiotic
873 resistance genes (ARGs) as a function of genetic lineage (defined by ML phylogeny),
874 time period and geography. A more detailed figure is provided in Supplementary Fig. 4.

875

876 **Figure 4. Evolution of antibiotic resistance of *Shigella dysenteriae* type 1. a**, Change
877 in the number of antibiotic resistance genes (ARGs) per isolate over time. The
878 logarithmic trendline and the correlation coefficient of determination (R^2) are shown in
879 red. **b**, Timeline of the first detection of the main ARGs in our collection. The antibiotics
880 (AMP, ampicillin; STR, streptomycin; SUL, sulfonamides; TMP, trimethoprim; CHL,
881 chloramphenicol; TET, tetracycline; NAL, nalidixic acid; and CIP, ciprofloxacin) for
882 which the ARGs convey resistance to are indicated. Asteriks indicate the mutation of
883 chromosomal genes of the core genome.

Figure 1.



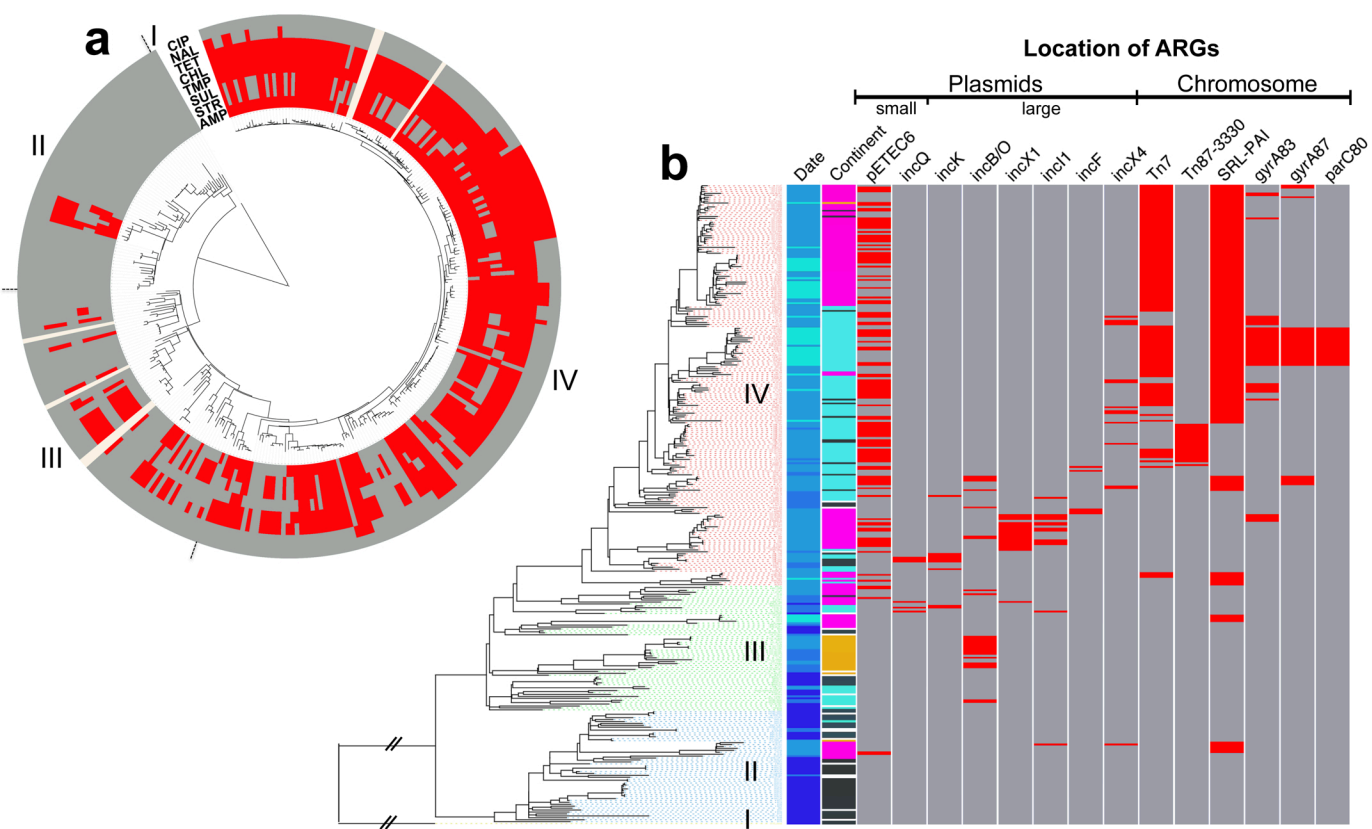


Figure 3.

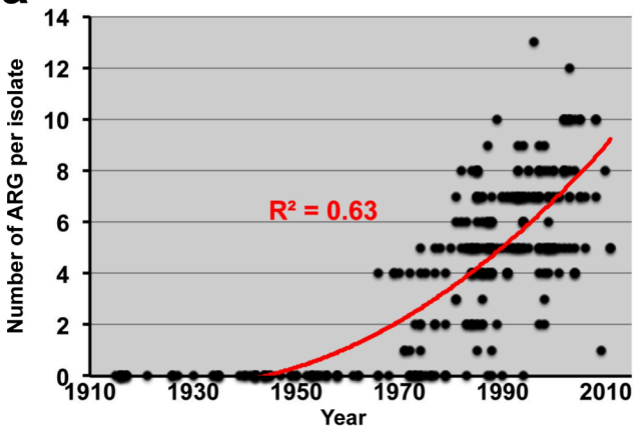
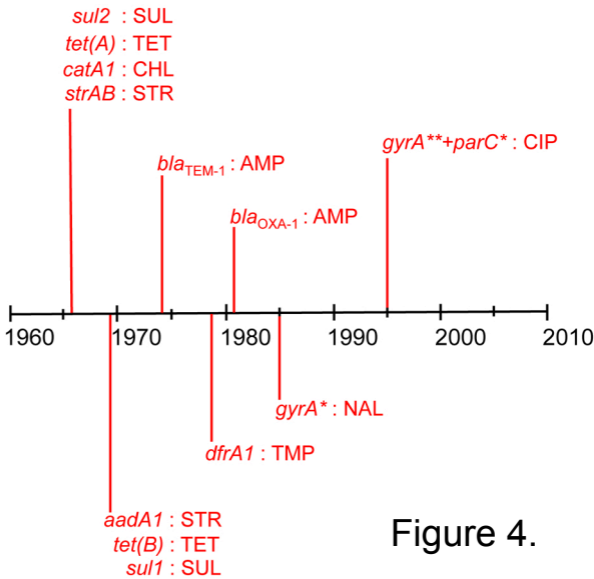
a**b**

Figure 4.