Global diversity and evolution of *Salmonella* Panama, an understudied serovar causing gastrointestinal and invasive disease worldwide: a genomic epidemiology study

Authors

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Abstract

Background

Nontyphoidal *Salmonella* (NTS) is a globally important bacterial pathogen, typically associated with foodborne gastrointestinal infection. Some NTS serovars can also colonise normally sterile sites in humans to cause invasive NTS (iNTS) disease. One understudied *Salmonella enterica* serovar which is responsible for a significant number of cases of iNTS disease is Panama. Despite global dissemination, numerous outbreaks, and a reported association with iNTS disease, *S. enterica* serovar Panama (*S.* Panama) has not been investigated in detail.

Methods

Using combined epidemiological and whole genome sequencing data we analysed 836 *S.* Panama genomes derived from historical collections, national surveillance datasets, and publicly available data. The collection represents all inhabited continents and includes isolates collected between 1931 and 2019. Maximum likelihood and Bayesian phylodynamic approaches were used to determine population structure & evolutionary history, and to infer geo-temporal dissemination. A combination of different bioinformatic approaches utilising short-read and long-read data were used to characterise geographic and clade-specific trends in antimicrobial resistance (AMR), and genetic markers for invasiveness.

Findings

We identified the presence of multiple geographically linked *S.* Panama clades, and regional trends in antimicrobial resistance profiles. Most isolates were pan-susceptible to antibiotics and belonged to clades circulating in the United States of America, Latin America, and the Caribbean. Multidrug resistant (MDR) isolates in our collection belonged to two phylogenetic clades circulating in Europe and Asia/Oceania, which exhibited the highest invasiveness indices based on the conservation of 196 extra-intestinal predictor genes.

55

56 **Interpretation**

57 This first large-scale phylogenetic analysis of *S.* Panama revealed important information about
58 population structure, AMR, global ecology, and genetic markers of invasiveness of the identified
59 genomic subtypes. Our findings provide an important baseline for understanding *S.* Panama
60 infection in the future. The presence of MDR clades with an elevated invasiveness index should be
61 monitored by ongoing surveillance as such clades may pose an increased public health risk.

62

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Research in context

Evidence before this study

Salmonella Panama has consistently been reported as a frequently isolated *Salmonella* serovar in national surveillance datasets, causing both sporadic cases and larger outbreaks. However, this picture has been masked due to most of the focus being placed on the top two serovars associated with nontyphoidal *Salmonella* (NTS) and invasive NTS disease: Typhimurium and Enteritidis. Previous works on *S.* Panama have determined transmission to include human faeces and breast milk, as well as non-human sources such as environmental reservoirs and animals (reptiles and pigs used for food). In contrast to most of the *Salmonella* serovars causing gastroenteritis, *S.* Panama has also been associated with a range of systemic infections including septicaemia, meningitis, and

osteomyelitis, mostly affecting infants. The patterns of antimicrobial resistance of this pathogen were not well known, with studies reporting a mixture of different antimicrobial resistance profiles.

Added value of this study

Here we conducted a large-scale study of 836 globally relevant *S.* Panama isolates to understand global population structure and disease ecology. The *S.* Panama genomes used in this study were sourced from a combination of historical collections (including the first ever isolated strain of this serovar dating from 1931), national surveillance datasets, and publicly available data. Some of these isolates had been linked to travel, allowing an inferred location for understanding population structure. Overall, this assembled *S.* Panama collection spanned a range of 88 years and covered 45 countries and regions of all inhabited continents. We applied bacterial phylodynamic approaches to determine population structure, evolutionary history, and clade-specific trends of invasiveness and antimicrobial resistance correlated with geo-temporal parameters.

Implications of all the available evidence

This study revealed the population structure and global ecology of *S.* Panama, indicating that multidrug resistant *S.* Panama circulates in European and Asian regions, and presents an increased number of genetic markers for extra-intestinal invasiveness. The transmission and expansion of antimicrobial-resistant *S.* Panama strains presented in this study highlight the significance of supporting control and monitoring efforts from an international perspective.

Introduction

Nontyphoidal *Salmonella* (NTS) disease poses a significant burden to public health globally, causing approximately 153 million cases and 57,000 deaths per annum. As well as being responsible for gastroenteritis in humans, NTS can also invade normally sterile body sites resulting in bacteraemia

130 and meningitis. There were estimated to be 535,000 cases of invasive NTS (iNTS) disease in 2017,
131 causing 77,500 deaths worldwide[1]. In humans, the clinical presentation depends upon a combination
132 of host immune factors and bacterial features that are specific to individual *Salmonella*
133 pathovariants[2]. The high levels of iNTS disease caused by *Salmonella enterica* serovars Typhimurium
134 and Enteritidis in sub-Saharan Africa have generated a significant research focus on these two
135 serovars[1]. However, little is known about other serovars, such as *S. enterica* serovar Panama
136 (hereafter referred to as *S.* Panama), which are also associated with iNTS disease and responsible for
137 numerous outbreaks, as reviewed previously[3].
138
139 Whilst the majority of cases of iNTS have been identified in sub-Saharan Africa[1,4], *S.* Panama has a
140 very different geographical distribution. *S.* Panama has consistently been reported as a leading cause
141 of NTS disease in Martinique, Guadeloupe, and French Guiana and is significantly associated with
142 causing invasive infections in children[3,5]. Although the proportion of salmonellosis cases caused by *S.*
143 Panama is high in these locations, the prevalence of antimicrobial resistance (AMR) is reportedly
144 low[3,5]. In contrast, other regions have seen higher levels of AMR in *S.* Panama[6]. For example, in the
145 1970s and 1980s *S.* Panama caused public health concern in Europe after spreading through the pork
146 industry and causing multiple hospital outbreaks[3]. *S.* Panama maintained its ranking as one of the
147 top 20 most frequently isolated serovars until 2017 in the European Union[7] and is a frequent cause
148 of invasive disease, with 7% of cases historically presenting as extraintestinal infection in England.
149 Similarly, *S.* Panama has been an important cause of iNTS in Asia, linked with high levels of AMR. Up
150 to 83% of domestic and imported *S.* Panama isolates in Tokyo were reported to be resistant to three
151 or more antimicrobial classes (multidrug resistant (MDR)) by the early 2000s[3]. In Oceania, *S.* Panama
152 was one of the top three serovars isolated between 2007 and 2016 in Queensland, Australia[8].
153
154 This study aimed to describe the genomic epidemiology and evolutionary history of *S.* Panama in
155 order to provide a vital baseline of understanding for this globally important serovar. We used whole

156 genome-based approaches on historical and contemporary global genomes to describe the
157 population structure of *S.* Panama using geographically and temporally diverse datasets. We
158 characterised four geographically-associated *S.* Panama clades and identified regional trends in
159 AMR. Our findings prompted a phylodynamic investigation which revealed the evolution of the
160 serovar. Finally, we determined genetic markers of invasiveness among *S.* Panama clades.
161

162 Methods

163 Study design

164 The *S.* Panama collection analysed in this study (*n* = 836) were derived from three public health
165 collections (the Unité des Bactéries pathogènes entériques (UBPE), French National Reference
166 Center for *Escherichia coli*, *Shigella*, and *Salmonella*, Institut Pasteur (Paris, France) (*n* = 559), the
167 Gastrointestinal bacteria reference unit, UK Health Security Agency (UKHSA, London, UK) (*n* = 147),
168 and the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL, Melbourne, Australia)
169 (*n* = 25) and a selection of publicly available genomes obtained through EnteroBase[9]
170 (http://enterobase.warwick.ac.uk) (*n* = 105). It was not possible to include all publicly available
171 genomes in our complete analysis due to the computational power required, thus we completed a
172 supplementary analysis of all publicly available genomes using Microreact to assess
173 representativeness and provide context (Figure S4, Supplementary Data 1, and
174 https://microreact.org/project/spanama-hc400-369).
175

176 A description of all the 836 isolates is available with all metadata and genome accession numbers in
177 Table S1 and Figure S5. Metadata collected from surveillance questionnaires was used to assign and
178 infer geographical location to each isolate. When a recent trip overseas was reported, the travel
179 destination was considered to be the inferred isolate location[10,11]. Where travel not reported, the
180 sampling location was taken to be the inferred location. Locations were grouped geographically into

181 six major areas based on the United Nations classification: Africa, Asia, Europe, Latin America & the
182 Caribbean, Northern America, and Oceania.
183
184 **Procedures**
185 Isolates from the Institut Pasteur were whole-genome sequenced either as part of the 10,000
186 *Salmonella* Genomes Project[12] (*n* = 322) using the Illumina HiSeq 4000 system or at the Institut
187 Pasteur (*n* = 237) using the NextSeq 500 system (Illumina). The *S.* Panama isolates 61-66, 86-66, and
188 376-66 were sequenced on a MinION Mk1C apparatus (Oxford Nanopore Technologies). Details
189 about DNA extraction and sequencing can be found in the Supplementary Methods.
190
191 To prepare genome sequence data for downstream analysis, reads were assembled, subjected to
192 quality control, and annotated as previously described[13]. The genomic sequences of isolates 61-66,
193 86-66, and 376-66 were assembled from long and short reads, with a hybrid approach and Unicycler
194 v0.4.8[14]. All the genome sequences are available via GenBank accession numbers listed in
195 Supplementary Table 1, and plasmids sequences under GenBank accession numbers OR797034
196 (pPan376-IncN), OR797033 (pPan86), OR797031 (pPan61-IncI1), and OR797032 (pPan61-IncN).
197
198 Trimmed sequencing data was mapped against *S.* Panama ATCC7378 as reference genome
199 (accession number CP012346) using Snippy v4.6.0 (https://github.com/tseemann/snippy) with
200 minimum coverage of 4 and base quality of 25. QualiMap[15] v2.0 was used to assess mapping quality
201 and only isolates with greater than 10x genome coverage were included in downstream analysis. The
202 mean coverage depth across all isolates was 45·13x. Snippy-core v4.6.0 was used to build a
203 reference-based pseudogenome for each isolate which was passed through snippy-clean v4.6.0 to
204 replace non-ACTG characters with "N". Gubbins[16] v2·2 was used to remove recombinant regions and
205 invariable sites. The resultant multiple sequence alignment of reference based pseudogenomes
206 (24,843 variant sites) was used to infer a maximum likelihood phylogeny using RAxML-NG[17] v1.0.3

207 with 100 bootstrap replicates to assess support. To assign clusters, RhierBAPs[18,19] was used
208 specifying two cluster levels, 20 initial clusters and infinite extra rounds. A maximum likelihood tree
209 was also constructed using RAxML-NG[17] for pPan61-IncN (alignment length = 47,695) and pPan376-
210 IncN (alignment length = 44,651) with isolates that had >50% coverage from C2.
211
212 To determine the evolutionary history of *S.* Panama, a chronogram was produced using BactDating[20]
213 v1.1. The maximum likelihood phylogenetic tree was used as an input, accounting for invariant sites.
214 BactDating's model comparison was used to determine the most appropriate clock model (mixed
215 arc). Effective sample size (ESS) were as follows: for mu = 501, sigma = 501, and alpha = 435. The
216 final model was run for 10,000,000 iterations to reach convergence. All phylogenies were visualised
217 using the Interactive Tree of Life (iTOL)[21] v4.2.
218
219 Genetic determinants for AMR were identified using staramr v0.5.1 (https://github.com/phac-
220 nml/staramr) against the ResFinder[22] and PointFinder[23] databases with default parameters (98%
221 identity threshold and 60/95% overlap threshold respectively). Common resistance patterns were
222 identified and the respective contigs were extracted from the assembled genomes. To understand
223 the genetic context of regions carrying AMR determinants, the contigs were manually inspected
224 using Artemis[24] v10.2. The Prokka-based annotations were confirmed and updated using BLASTx[25]
225 v2.10.1 to compare all coding regions against the non-redundant protein sequence database.
226 PlasmidFinder[26] was used to predict contigs containing plasmids, and the results were compared
227 against contigs containing AMR determinants. Finally BLASTn[25] v2.10.1 was used to understand the
228 wider context of the genetic region.
229
230 The invasiveness index of each isolate was calculated using previously defined methods, using the
231 196 top predictor genes for measuring invasiveness of *S. enterica* provided in Wheeler *et al.*

232 (2018)[27]. We used the same pre-trained model to compare the median invasiveness index for *S.*
233 Panama with the invasiveness index of validation strains previously described[27].
234
235 **Statistical analysis**
236 The distribution of invasiveness index values for each cluster were compared using the Kruskall-
237 Wallis test implemented through GraphPad Prism v10.1. SRST2[46] v0.2.0 was used to identify
238 variations between the 196 genes in study isolates.
239
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241 The funders of the study had no role in study design, data collection, data analysis, data
242 interpretation, or writing of the report.
243

244 Results

245 To create a relevant dataset, we sequenced the genomes of 552 *S.* Panama isolates, and included an
246 additional 284 *S.* Panama genomes from a combination of historical collections, national surveillance
247 datasets, and publicly available data, including the first isolate obtained in Panama in 1931 during a
248 food poisoning outbreak in US soldiers stationed in the Canal zone (Figure 1, Table S1, and Figure
249 S5). The majority (67% n = 559) of the isolates were sourced from the Institut Pasteur, Paris, France.
250
251 For public health datasets, an isolate was deemed to be "travel associated" if a case had reported
252 travel up to 28 days (UKHSA), seven days (MDU PHL) or 2 months (Institut Pasteur) prior to symptom
253 onset. The "inferred region" of each isolate was set as the travel destination where available (n = 95)
254 or kept as the isolation region (n = 741).
255

256 Overall, the collection of 836 *S*. Panama included 771 human isolates of which 403 were isolated
257 from stool, 290 were derived from extra-intestinal sites (blood and cerebrospinal fluid), and 78 were
258 taken from other body sites (Figure 1, Table S1). Of the 65 non-human isolates, six were of
259 environmental origin (mainly water sources), 22 were obtained from food, 30 were from livestock,
260 four were from wild animals, and three were of unknown origin (Supplementary Table S1). These
261 isolates cluster with the region of origin rather than within their source group. Further work should
262 be considered for addressing animal and environmental reservoirs.
263
264 To define population structure, we explored the phylogenetic relationship among the 836 *S.* Panama
265 genomes and defined population groupings using Rhier Bayesian Analysis of Population Structure
266 (RhierBAPS)[18,19] (Figure 2). The BAPS groupings reflected four monophyletic clades with greater than
267 95% bootstrap support containing variable numbers of isolates. We defined the four clades as C1 (*n*
268 = 338), C2 (*n* = 124), C3 (*n* = 131), and C4 (*n* = 104). The remaining 139 isolates were not assigned to
269 a clade due to the level of diversity. Temporal regression of the phylogeny showed that while the
270 inferred substitution rates were comparable among Clades C1 – C3, Clade C4 evolved nearly an
271 order of magnitude faster (Figure S6, Table S2).
272
273 To investigate the relationship between clades and geographic region, location data was correlated
274 with phylogenetic position (Figure 2). Where information on travel was available, we used the
275 destination of travel as an inferred location. All the travel-associated isolates came from the Institut
276 Pasteur's national surveillance dataset (*n* = 43), UKHSA (*n* = 42), and MDU PHL (*n* = 18), leading to
277 the inclusion of a further 12 countries not previously represented by isolates.
278
279 The majority (*n* = 295/338) of genomes clustering in clade C1 were from Latin America & the
280 Caribbean, including the first *S.* Panama isolate from 1931. Within Clade C1, there were five
281 geographically-linked monophyletic clusters: three were dominated by isolates from Guadeloupe,

282 Martinique, and French Guiana respectively, and two contained isolates from multiple geographical
283 locations in Latin America & the Caribbean (Figure 2). Clade C1 was named the "Latin America & the
284 Caribbean clade" reflecting its geographical composition. A further clade, Clade C3 consisted of
285 isolates from Martinique (*n* = 125/131), and was named the "Martinique clade".
286
287 Clade C2 was mainly composed of isolates obtained from Europe, and included distinct subclades in
288 which isolates from metropolitan France and the UK were overrepresented. Specifically, while the
289 majority (84% *n* = 107/124) of isolates in each of these subclades came from the UK (*n* = 60/124) and
290 metropolitan France (*n* = 47/124), C2 also contained isolates from less well sampled European
291 countries including Germany, Malta, Ireland, and the Netherlands. These findings are consistent with
292 European-wide circulation, and so C2 was designated as the "European clade".
293
294 Clade C4 included isolates mainly from Asia and Oceania (*n* = 63/104). We identified three C4
295 subclades, of which two were dominated by isolates obtained from Asia and Oceania and a third
296 comprised of isolates obtained in the USA. Accordingly, C4 was termed the "Asia/Oceania clade".
297
298 The majority of genomes obtained from travel associated cases were related to travel to Asia (*n* =
299 52) and Latin America & the Caribbean (*n* = 36), which mainly cluster within C4 (Asia) and C1 & C3
300 (Latin America & the Caribbean). To assess representativeness, we placed our collection into the
301 context of all 3,051 publicly available *S*. Panama genomes (using core genome multilocus sequence
302 typing hierarchical cluster HC400 = 369, as of October 2024) in EnteroBase[9] and completed a
303 supplementary analysis using Microreact (Figure S4, Supplementary Data 1, and
304 https://microreact.org/project/spanama-hc400-369). This comparison suggests that our collection
305 provides good representation of publicly accessible data.
306

307 Of the 836 isolates, 14% (*n* = 121/836) were predicted bioinformatically to be resistant to at least
308 one antimicrobial class. Previous studies have demonstrated that genome-based AMR analysis
309 accurately predicted phenotype for 98% of *Salmonella* isolates[28]. The remaining 86% (*n* = 715/836)
310 isolates were predicted to be pan-susceptible, lacking any known antibiotic resistance genes or
311 mutations. Most resistant isolates in our collection (*n* = 113/121) fell within C2 (European clade) and
312 C4 (Asia/Oceania clade) (Figure 3). A search of publicly available *S.* Panama genomes revealed a
313 small number of MDR isolates sourced in the US. The majority of the MDR US isolates were
314 phylogenetically situated with isolates in our collection that belonged to C4 (Asia/Oceania clade) or
315 had clade unassigned (Supplementary Figure S4, Supplementary Data 1, and
316 https://microreact.org/project/spanama-hc400-369).
317
318 The most common resistance profile, occurring in 36% (*n* = 43/121) of resistant isolates, was against
319 streptomycin (*aadA1, aadA2*), ampicillin (bla_{TEM-1B}), chloramphenicol (*cmlA1*), trimethoprim (*dfrA12*),
320 sulfisoxazole (*sul3*), and tetracycline (*tetA*). Detailed analysis of the draft genomes showed that most
321 of the resistance genes (*aadA1*, *aadA2*, *cmlA1*, *dfrA12* and *tetA*) were encoded by an MDR cassette
322 located on a single contiguous sequence (contig; Figure 3B). A BLAST search of the contig revealed
323 99·8% sequence identity to a 21·2 kb region of an 83·2 kb plasmid (GenBank accession number
324 CP044301) which had previously been identified in an *E. coli* isolate obtained from pork in
325 Cambodia[29].
326
327 The second most common AMR profile, found in 12% (15/121) of resistant isolates was against
328 streptomycin (*aph(3'')-Ib* and *aph(6)-Id*), ampicillin (bla_{TEM-1B}), trimethoprim (*dfrA14*), sulfisoxazole
329 (*sul2*), and tetracycline (*tetA*). All genes were carried by an MDR cassette located on a single contig
330 (Figure 3B). The contig shared 99·9% sequence identity with a 28·3 kb region of a 50·9 kb *S. enterica*
331 plasmid (GenBank accession number CP028173), which has been identified in multiple strains
332 including an *S.* Enteritidis isolate from Ghana[30].

333

334 The remaining resistant *S.* Panama isolates had a variety of susceptibility profiles, including one
335 extensively drug resistant (XDR) isolate 201209115 (GenBank accession number SAMEA6142076),
336 which was phylogenetically situated within C4 (Asia/Oceania clade) and was isolated in 2012 from
337 the bloodstream of a case who had reported recent travel to Thailand. The nomenclature used to
338 describe the XDR isolate is in line with previous definitions of resistance to three first-line drugs
339 (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) as well as fluoroquinolones and
340 third-generation cephalosporins[31]. The isolate carried AMR determinants against eight different
341 antimicrobial classes, including: fluoroquinolones, polymyxins, and extended spectrum beta-
342 lactamase (ESBL) mediated resistance to third generation cephalosporins ($bla_{CTX\text{-}M\text{-}55}$). The resistance
343 genes in the XDR isolate were distributed across five contigs (Figure S1), with the kanamycin
344 (*aph(3')-Ia*) and gentamicin (*aac(3)-IId*) genes being carried on separate contigs. Genes encoding
345 resistance to streptomycin (*aadA1, aadA2*), chloramphenicol (*cmlA1*), trimethoprim (*dfrA12*), and
346 sulfisoxazole (*sul3*) were co-located on the same contig, and the resistance genes against
347 ciprofloxacin (*qnrS1*), ampicillin, and ceftriaxone ($bla_{CTX\text{-}M\text{-}55}$) were also co-located on a separate
348 contig.

349

350 Due to resource constraints, it was not possible to conduct a contig-based analysis for all isolates,
351 consequently a short-read based analysis was initially conducted followed by targeted resequencing
352 with Oxford Nanopore. Our short read-based plasmid prediction analysis identified that only 16% (*n*
353 = 131/836) of *S.* Panama isolates in this study carried plasmids, including 65% (*n* = 85/131) of
354 resistant isolates and 35% (*n* = 46/131) susceptible isolates. The main plasmid incompatibility groups
355 identified were IncFIA(HI1) (*n* = 40/131) and IncFIB(K) (*n* = 35/131) restricted to C4 (Asia/Oceania
356 clade), with the main AMR profile streptomycin, ampicillin, chloramphenicol, trimethoprim,
357 sulfisoxazole, and tetracycline (*n* = 27/35). IncN plasmids were identified mostly in C2 (European

358 clade) (*n* = 28/131) that shared the same AMR profile as IncFIA(HI1) and IncFIB(K) plasmids, but
359 includes kanamycin resistance instead of chloramphenicol resistance.
360
361 To explore AMR context and evolution within C2 (which shows high levels of resistance), plasmid
362 DNA of three MDR isolates (isolates 61-66, 86-66, and 376-66) were extracted and sequenced using
363 long-read sequencing technology (Oxford Nanopore). These isolates were collected during hospital
364 outbreaks caused by *S*. Panama in France in 1966 and clustered within C2 (European clade).
365 Sequence analysis showed a 45 kb IncN plasmid (pPan376-IncN) encoding resistance to tetracycline
366 (*tetA*), and a small 8 kb plasmid (pPan86) encoding resistance to ampicillin (bla_{TEM-1}). Functional
367 annotation of the plasmids identified in isolate 61-66 showed a 104 kb IncI1 plasmid (pPan61-IncI1)
368 with integration of the bla_{TEM-1} gene, and a 48 kb IncN plasmid (pPan61-IncN) encoding tetracycline
369 and ampicillin resistance, potentially from the fusion of pPan376-IncN and pPan86 (Figure S2).
370
371 To understand whether these IncN plasmids are driving resistance in recent European clade C2
372 isolates, we screened for the presence of plasmids in the genomes of all C2 isolates. This screening
373 indicated that 23% (*n* = 27/124) of isolates contained pPan376-IncN, 26% (n = 32/124) contained
374 pPan86, 2% (*n* = 3/124) pPan61-IncI1, and 23% (*n* = 28/124) pPan61-IncN (Figure S2 and Table S1).
375 We constructed phylogenies of the two most prevalent IncN plasmids to explore coevolution and
376 determine whether these plasmids are continuing to drive current AMR in *S.* Panama (Figure S2).
377 The results show temporal clustering, indicating that the older and contemporary IncN plasmids are
378 distinct.
379
380 To investigate the evolutionary dynamics of the global *S*. Panama population, we inferred the times
381 to most recent common ancestor (MRCA) of each clade using BactDating (Figure 4)[20]. Analysis of the
382 chronograph predicted the MRCA of all isolates in this study to be year 1555 with a highest
383 probability density interval (HPD) of 1458 to 1634. C1 (Latin America & the Caribbean clade) had an

384 MRCA of 1899 (HPD 1880-1911) with separate introductions into Guadeloupe, Martinique, and
385 French Guiana, followed by clonal expansion in the 1930s and 1950s in each of the three regions.
386 The MRCA of C2 (European clade) was 1879 (HPD 1852-1894), followed by expansion of more
387 contemporary subclades in the 1970s. The MRCA of C3 (Martinique clade) is 1880 (HPD 1862-1916),
388 and of C4 (Asia/Oceania clade) is 1879 (HPD 1854-1905). These predicted timeframes overlap with
389 European attempts to, and the final construction of, the Panama Canal.
390
391 Whilst it was beyond the scope of this study to provide a detailed analysis of all possible markers of
392 invasiveness, we provide a snapshot of the invasive potential of *S.* Panama using the "invasiveness
393 index" model[27] (Table S1). The model provides a prediction of invasiveness by assessing genome
394 degradation within 196 genes and has been validated for other *Salmonella* serovars[27]. The mean
395 invasiveness index of *S.* Panama was 0·2294 (SD = 0·01790). Within *S.* Panama, C2 had the greatest
396 mean invasiveness index (European clade; median (IQR) was 0·2474 (0·2336-0·2502), followed by C3
397 (Martinique clade; median (IQR) was 0·2343 (0·2229-0·2367), C1 (Latin America & the Caribbean
398 clade; median (IQR) was 0·2303 (0·2210-0·2408), and C4 (Asia/Oceania clade; median (IQR) was
399 0·2283 (0·2186-0·2407)).
400

401 ## Discussion

402 This study has described the genomic epidemiology and evolutionary history of *S.* Panama, providing
403 a vital baseline of understanding for this globally important serovar with relevance to genome-based
404 surveillance internationally. Our finding revealed the presence of four geographically-linked
405 phylogenetic clades, with C1 consisting predominantly of isolates from Latin America & the
406 Caribbean, C2 from Europe, C3 from Martinique and C4 from Asia/Oceania.
407

408 The majority of *S.* Panama in this study were pan-susceptible to antibiotics, however there are a
409 number of MDR isolates in C2 (European clade) and C4 (Asia/Oceania clade) (Figure 3), of which the
410 former presented genomic predictors of elevated invasiveness (Figure S3B). These findings are
411 supported by reports of an increase in *S.* Panama isolates from Asia that were resistant to multiple
412 antibiotics, including cotrimoxazole, ampicillin, streptomycin, kanamycin, and gentamicin[32].
413
414 We also identified clustered AMR elements on the genome that are consistent with plasmid-
415 mediated resistance in *S.* Panama, as has been seen in previous studies[32]. The plasmid complement
416 of the *S.* Panama serovar has rarely been studied in the past, but it has been reported that *S.*
417 Panama does not commonly carry the large virulence plasmids that have previously been
418 characterised in other *Salmonella* serovars[33]. Rather, *S.* Panama have previously been found to carry
419 a heterogeneous population of plasmids (Table S1)[34].
420
421 The evolutionary dynamics analysis of the global *S.* Panama population provided information about
422 the MRCA of each clade (Figure 4). The four clades were predicted to share a most recent common
423 ancestor in the 1500s and have undergone contemporary introductions into different geographical
424 regions. We identified isolates from both Guadeloupe and Martinique in C1 and C3, suggesting that
425 inter-island transmission of C1 is likely occurring. We speculate that C2 arose from an outbreak that
426 involved the continental spread of *S.* Panama across Europe, associated with the food production
427 industry in the 1970s and 1980s[3]. It is possible that these European *S.* Panama were exposed to
428 significant levels of antibiotics in humans and/or food animals, which selected for resistance to
429 multiple antibiotic agents via acquisition of mobile genetic elements[3], consistent with the indicators
430 of mobilisation (Figure 3). The emergence of C2 (European clade) in the 1970s is consistent with this
431 hypothesis, coinciding with the propagation of *S.* Panama in Europe via the pig industry[3]. To note,
432 we identified an MDR isolate from swine in the US (SRR8381713) as part of our analysis of all

433 publicly available data. However, this isolate was phylogenetically situated in C4 (Asia/Oceania
434 clade).
435
436 Although *S.* Panama causes extraintestinal disease globally, genetic determinants of invasiveness
437 have never been investigated. In this context, invasiveness reflects the ability of the pathogen to
438 cause systemic spread and extraintestinal infection in humans[35]. Invasive infections cause much
439 higher levels of mortality than gastroenteritis; for example, the case-fatality rate of iNTS is 20%[1],
440 whereas the case-fatality rate of *S.* Typhimurium-associated gastroenteritis is 0·6%[36]. It has been
441 observed that functions required for escalating growth within the inflamed gut are often lost among
442 iNTS variants in a series of gene degradation/pseudogenisation events[13]. The increased clinical
443 invasiveness observed amongst *S.* Panama is likely influenced by several factors, related to both the
444 host (e.g. immunocompetency) and pathogen (e.g. presence/absence of genes that elevate
445 invasiveness). The calculated invasiveness index of *S.* Panama is slightly higher than that described
446 for serovars with a typically broader host range (Agona, Enteritidis, Heidelberg, Newport, and
447 Typhimurium)[27] but significantly lower than that of host-restricted serovars (Typhi, Paratyphi,
448 Gallinarum, Pullorum, Dublin, and Choleraesuis)[27] (Figure S3A). Public health surveillance should
449 take into consideration the significantly higher invasiveness index of C2 (European clade) combined
450 with the high proportion of AMR isolates when risk assessing outbreaks falling within this clade
451 (Figure S3B).
452
453 There are limitations associated with travel-related data in this study. For example, it is possible that
454 cases who reported travel did not necessarily acquire their infection whilst travelling. Furthermore,
455 information on travel was only available for public health datasets with limited (likely under-
456 reported) or unavailable travel information for all cases. Thus, there are likely some geographical
457 signals missing which may impact the interpretation of geographically-linked clade designations.
458 However, we note that for all four clades in which a geographical designation has been made, there

459 are a substantial number of isolates from the relevant region. This is indicative of domestic
460 circulation, but does not totally exclude the possibility that isolates were initially imported via travel.
461 Moreover, our analysis only shows the population structure of those isolates selected for inclusion in
462 this *S.* Panama collection. Thus, the analysis may be subject to a number of sampling biases. Isolates
463 submitted to national surveillance teams at public health centres will be limited to those sampled
464 from cases who attend healthcare services and have a sample collected, which may favour more
465 clinically severe cases. Also, not all isolates submitted to national surveillance will be whole-genome
466 sequenced and uploaded to publicly available repositories, with approaches differing by country.
467 The publicly available datasets may also include isolates submitted as part of research projects, each
468 of which will have had its own sampling strategy that may have favoured sequencing of isolates with
469 specific characteristics, for example, those with specific antimicrobial resistance patterns. Finally, the
470 analysis is limited by the sampling strategy which purposively selected for temporal and
471 geographical breadth. As a result, our analysis likely captures only a partial view of the true global
472 population structure of *S.* Panama.
473
474 As the first large-scale phylogenetic analysis of the *S.* Panama serovar to our knowledge, we have
475 revealed important information about population structure, AMR, global ecology, and invasiveness.
476 It will be important to monitor the incidence of clades C2 and C4 in ongoing genome-based
477 surveillance, and to understand the movement of mobile genetic elements responsible for antibiotic
478 resistance. It is possible that the evolutionary trajectory of *S*. Panama will parallel the *Salmonella*
479 serovars Enteritidis and Typhimurium, which contain specific lineages and pathovariants with an
480 increased invasiveness index and are now causing significant levels of human bloodstream
481 infections[1,27,37]. Future work on *S.* Panama can be derived from the current knowledge of serovars
482 Enteritidis and Typhimurium and should focus on understanding the molecular basis of systemic
483 invasion, the development of a robust typing system, and a defined animal infection model.
484

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Declaration of interests

The authors declare no conflict of interest.

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589

590 ## Figure and Table legends

591 **Figure 1. The sample collection used to construct the *S.* Panama population structure.**

592 (A) Total number of isolates per collection. Stacked colour bars on the left are proportional to the
593 number of isolates from that source, and colours act as a key for histograms to indicate source.
594 Airplane symbols show the number of travel-associated isolates obtained by the Institut Pasteur and
595 United Kingdom Health Security Agency (UKHSA). Three stacked histograms show the number of
596 isolates from UKHSA, Institut Pasteur, and MDU PHL (upper graph), publicly available data (middle
597 graph), and historical collections (lower graph). (B) Number of isolates per region (ambiguous or
598 outdated locations registered as "Caribbean", "Asia", "Antilles", and "Yugoslavia" are omitted from
599 the map), with regions shaded according to the inlaid key. Map created with MapChart
600 (http://mapchart.net). (C) Number of isolates categorised by source and, for human isolates,
601 specimen type. Three isolates of unknown source are indicated in grey. Shape sizes are proportional
602 to the number of isolates.

603

604 **Figure 2. Population structure and geographical distribution of *S.* Panama isolates**

605 Maximum likelihood phylogenetic tree of 837 isolates (including reference isolate ATCC7378 marked
606 by a purple arrow) showing clade (first column) and subclade (second column) assignment based on
607 RhierBAPS, inferred region (third column), and number of isolates (fourth column) as a graph with
608 countries (x-axis) clustered and coloured by the United Nations geographical regions. Data for
609 isolates not assigned to a clade due to the level of diversity were labelled as "other" (asterisked) and
610 were combined into a single (asterisked) histogram marked in grey.

611

612 **Figure 3. Antimicrobial resistance trends in *S.* Panama**

613 (A) Maximum likelihood phylogenetic tree showing clades (first column), inferred region (second
614 column) coloured based on UN geographical regions, AMR (third column) presence of at least one
615 resistance conferring gene (red) or absence (grey), and presence of predominant AMR profiles: AMR

616 profile 1 (green) and profile 2 (pink). (B) Number of isolates (coloured by clade) with different AMR
617 profiles. Resistance to different antimicrobials is shown as present (dark circles) or absent (light
618 circles). Dark circles are connected to highlight a variety of unique AMR profiles. The two
619 predominant AMR profiles (AMR profile 1: resistance to ampicillin, streptomycin, tetracycline,
620 trimethoprim, and chloramphenicol, and AMR profile 2: resistance to ampicillin, streptomycin,
621 tetracycline, trimethoprim, and kanamycin) are highlighted. Functional annotation of contiguous
622 sequences with the two predominant AMR profiles (respectively indicated by grey arrows pointing
623 to sequence schematics). Arrows indicate predicted reading frames highlighting genes encoding
624 AMR (red) and transposases (light blue).
625
626 **Figure 4. Phylodynamic and evolutionary timescales of *S.* Panama**
627 Chronograph for estimation of most recent common ancestors for *S*. Panama clades. Arrows indicate
628 MCRA for each clade, with year and highest probability density (95%) interval shown as text.
629

630 ## Appendix:

631 **Supplementary Methods**
632 **Supplementary Data 1: Microreact project of All *S. enterica* HC400 = 369 available in EnteroBase**
633 **Table S1: *S.* Panama metadata and accession numbers**
634 **Table S2: Nucleotide substitution analysis of four *S.* Panama clades**
635
636 **Figure S1: Functional annotation of five AMR gene cassettes in XDR isolate 201209115**
637 The predicted phenotype conferred (left) and total length (right) for each contiguous sequence that
638 carries an AMR gene cassette is displayed. Arrows indicate open reading frames, and AMR genes are
639 highlighted in red. GenBank accession number SAMEA6142076.
640

641 **Figure S2: Conservation and functional annotation of plasmids within C2 ascertained with long-**
642 **read sequencing technology**
643 (A) Maximum likelihood phylogenetic tree of C2 showing subclades (column one), inferred region
644 (column two) of isolation coloured by UN geographical regions in Figure 2, year (column three), AMR
645 (column four) presence (red) or absence (grey), and percentage coverage (>50%) of pPan61-IncI1
646 (column five), pPan61-IncN (column six), pPan376-IncN (column seven), and pPan86 (column eight).
647 (B) Pairwise comparison of linearised plasmids showing genomic similarity (red) of pPan376-IncN
648 and pPan86 with pPan61-IncN. GenBank accession numbers are shown below the plasmid name. (C)
649 Phylogenetic trees of pPan376-IncN and pPan61-IncN showing year of isolation (first column) and
650 AMR (following column(s)). The AMR profile of all isolates in pPan376-IncN tree was streptomycin,
651 ampicillin, chloramphenicol, trimethoprim, sulfisoxazole, tetracycline. The sequenced plasmids that
652 acted as reference sequences are indicated by a star. (D) Functional annotation of genes on pPan61-
653 IncI1, pPan61-IncN, pPan376-IncN, and pPan86.
654
655 **Figure S3: Invasiveness index of *S.* Panama clades in comparison with common *S. enterica* serovars**
656 (A) Median invasiveness index of different *Salmonella* serovars as calculated in Wheeler et al (2018)
657 (black dots) with *S.* Panama (this study) highlighted in pink. (B) Distribution of invasiveness index of
658 all 836 isolates clustered by clade. Overlying box and whiskers plots indicate median and
659 interquartile range, which are enumerated below in the table. Comparisons between groups are
660 indicated by lines uppermost with *p*-values indicated above each line.
661
662 **Figure S4: All *S. enterica* HC400 = 369 available in EnteroBase**
663 Screenshot of Microreact project containing the data publicly available (October 2024) in EnteroBase
664 (https://enterobase.warwick.ac.uk/) for all *S.* Panama genomes identified (search criteria:
665 experimental data HC400 = 369, total of 3,051 assembled genomes) accessible in
666 https://microreact.org/project/spanama-hc400-369 and Supplementary Data 1.

667

668 **Figure S5: *S.* Panama phylogenetic tree with annotated tips**

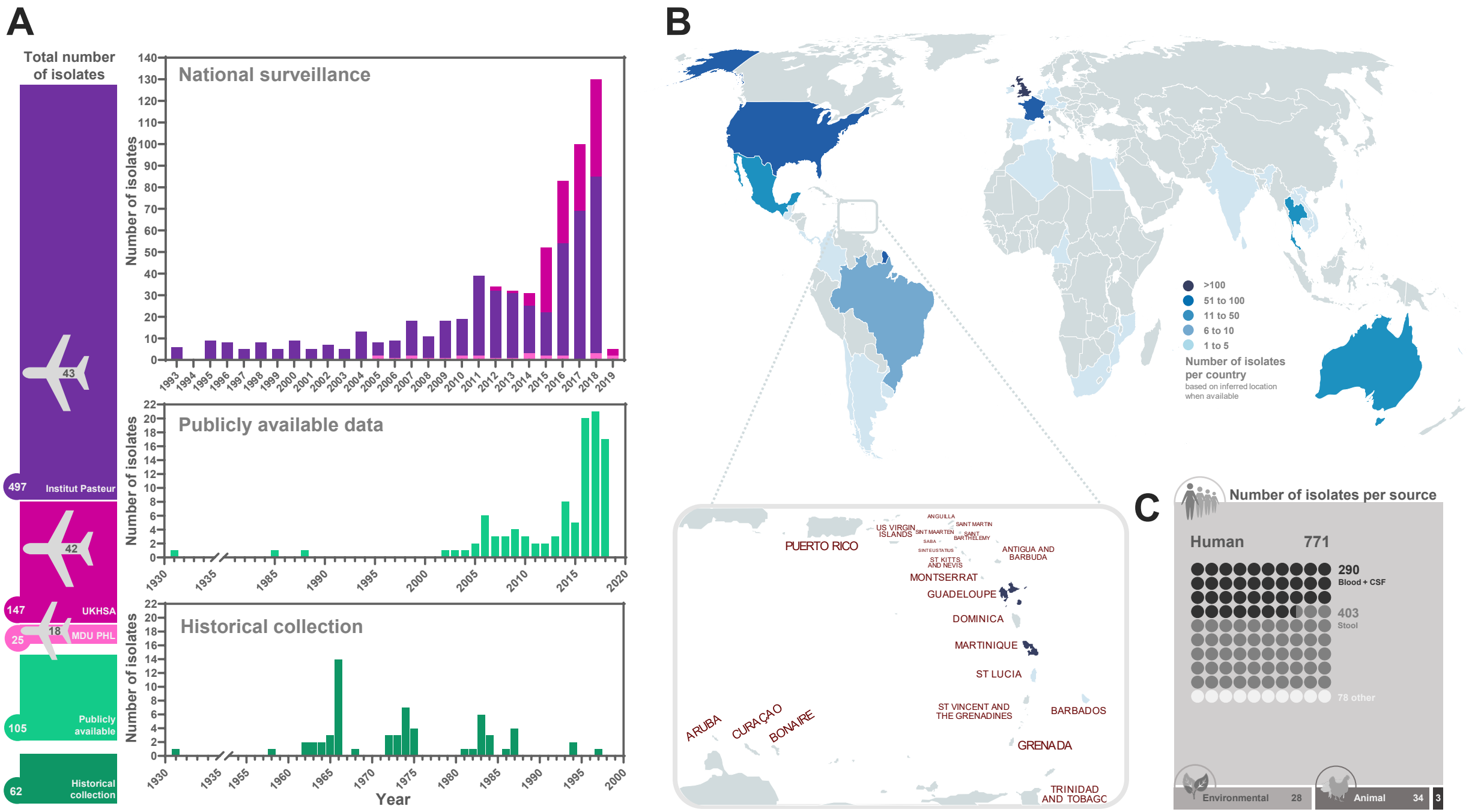
669 High resolution maximum likelihood phylogenetic tree showing clades (first column), inferred region
670 (second column) coloured based on UN geographical regions, and AMR (third column) presence of at
671 least one resistance conferring gene (red) or absence (grey). All genome accession numbers are
672 annotated in tree tips, corresponding with data available in Supplementary Table S1.

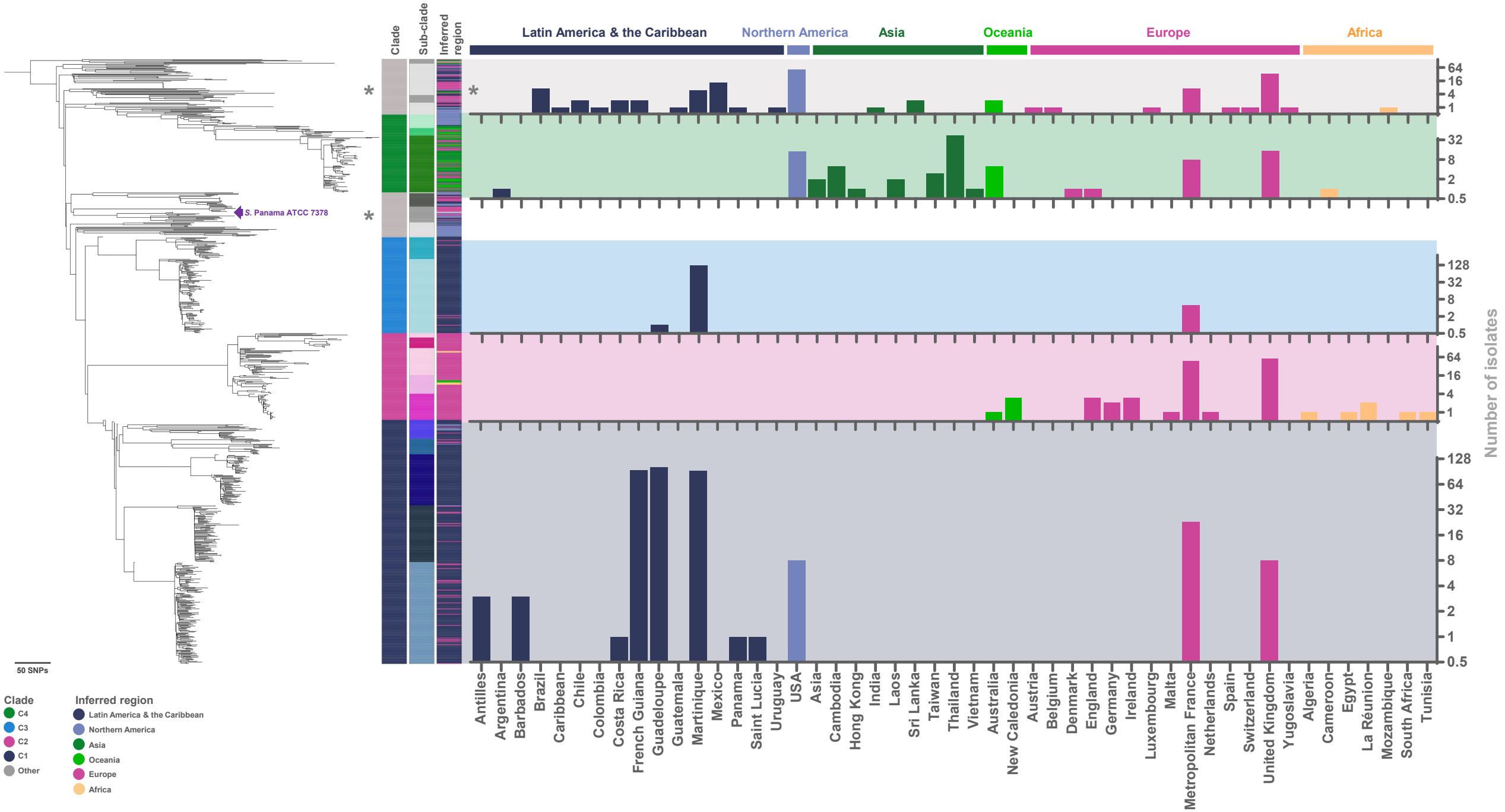
673

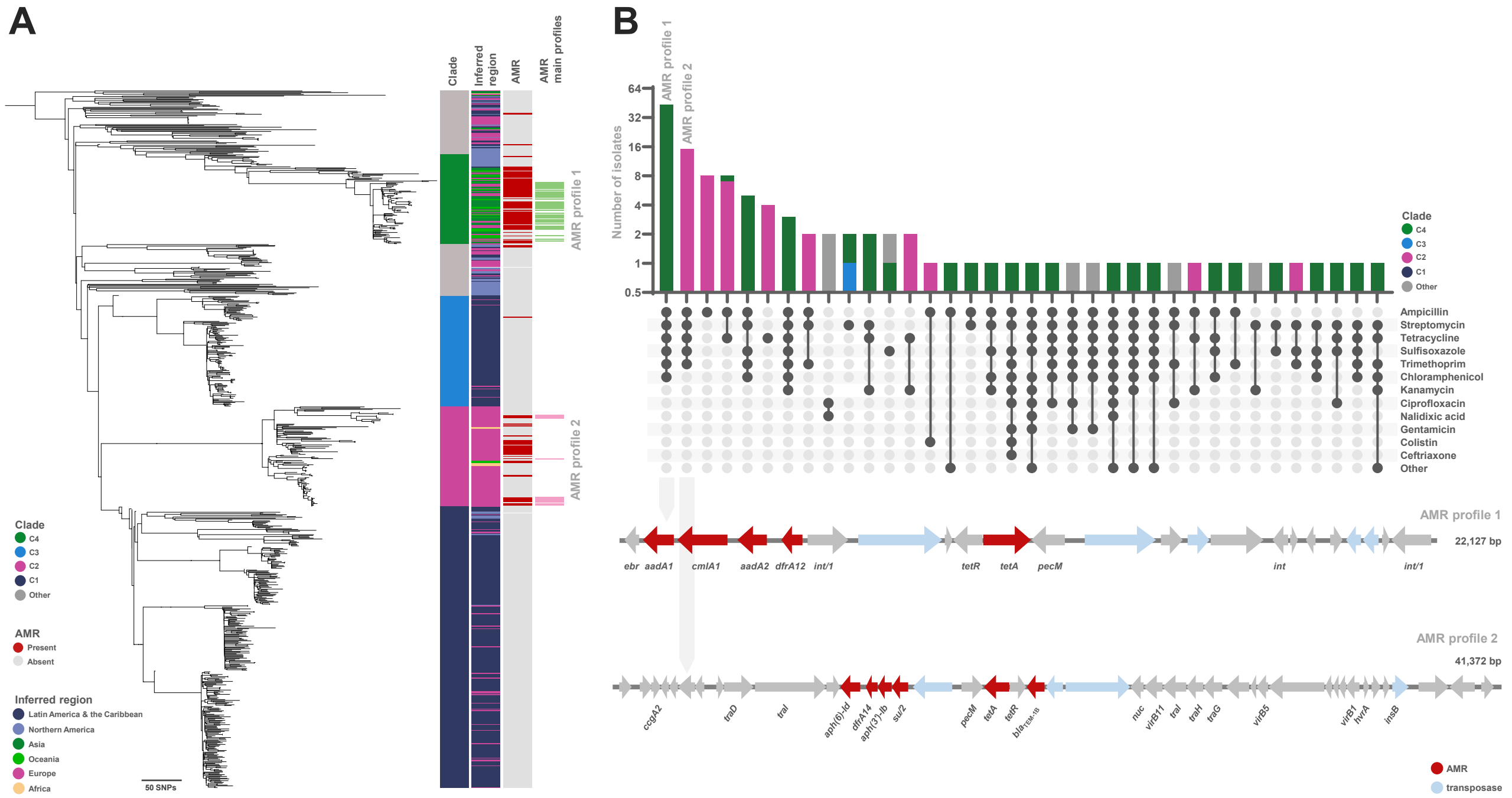
674 **Figure S6. Nucleotide substitution analysis of four *S.* Panama clades.**

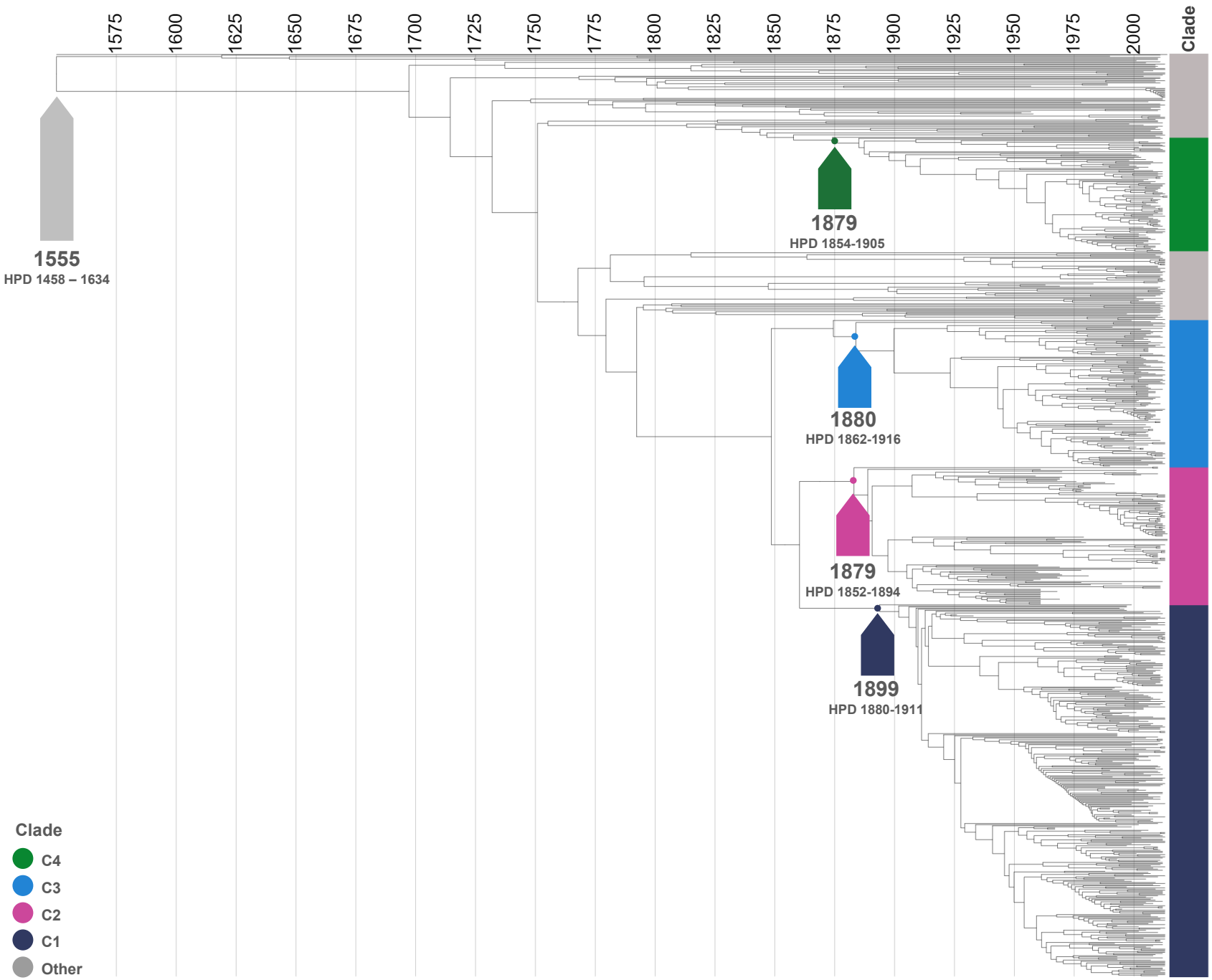
675 Root-to-tip distance extracted from the maximum likelihood phylogenetic tree of 837 isolates
676 (excluding reference isolate ATCC 7378) plotted against year of isolation. Data available in
677 Supplementary Table S2.

678


A
Total number of isolates
497
Institut Pasteur
43
147
UKHSA
42
25
MDU PHL
18
105
Publicly available
62
Historical collection
National surveillance
Publicly available data
Historical collection
Number of isolates
Year
B
>100
51 to 100
11 to 50
6 to 10
1 to 5
Number of isolates per country
based on inferred location when available
PUERTO RICO
US VIRGIN ISLANDS
ANGUILLA
SAINT MARTIN
SINT MAARTEN
SAINT BARTHELEMY
SABA
SINT EUSTATIUS
ST KITTS AND NEVIS
ANTIGUA AND BARBUDA
MONTSERRAT
GUADELOUPE
DOMINICA
MARTINIQUE
ST LUCIA
ST VINCENT AND THE GRENADINES
BARBADOS
GRENADA
TRINIDAD AND TOBAGO
ARUBA
CURAÇAO
BONAIRE
C
Number of isolates per source
Human 771
290
Blood + CSF
403
Stool
78 other
Environmental 28
Animal 34
3


Clade
Sub-clade
Inferred region
Latin America & the Caribbean
Northern America
Asia
Oceania
Europe
Africa
S. Panama ATCC 7378
50 SNPs
Number of isolates
Antilles
Argentina
Barbados
Brazil
Caribbean
Chile
Colombia
Costa Rica
French Guiana
Guadeloupe
Guatemala
Martinique
Mexico
Panama
Saint Lucia
Uruguay
USA
Asia
Cambodia
Hong Kong
India
Laos
Sri Lanka
Taiwan
Thailand
Vietnam
Australia
New Caledonia
Austria
Belgium
Denmark
England
Germany
Ireland
Luxembourg
Malta
Metropolitan France
Netherlands
Spain
Switzerland
United Kingdom
Yugoslavia
Algeria
Cameroon
Egypt
La Réunion
Mozambique
South Africa
Tunisia
Clade
C4
C3
C2
C1
Other
Inferred region
Latin America & the Caribbean
Northern America
Asia
Oceania
Europe
Africa


A
Clade
Inferred region
AMR
AMR main profiles
AMR profile 1
AMR profile 2
Clade
C4
C3
C2
C1
Other
AMR
Present
Absent
Inferred region
Latin America & the Caribbean
Northern America
Asia
Oceania
Europe
Africa
50 SNPs
B
AMR profile 1
AMR profile 2
Number of isolates
64
32
16
8
4
2
1
0.5
Clade
C4
C3
C2
C1
Other
Ampicillin
Streptomycin
Tetracycline
Sulfisoxazole
Trimethoprim
Chloramphenicol
Kanamycin
Ciprofloxacin
Nalidixic acid
Gentamicin
Colistin
Ceftriaxone
Other
AMR profile 1
22,127 bp
ebr
aadA1
cmlA1
aadA2
dfrA12
int/1
tetR
tetA
pecM
int
int/1
AMR profile 2
41,372 bp
ccgA2
traD
traI
aph(6)-Id
dfrA14
aph(3')-Ib
sul2
pecM
tetA
tetR
bla TEM-1B
nuc
virB11
traI
traH
traG
virB5
virB1
hvrA
insB
AMR
transposase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1600
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1800
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1900
1925
1950
1975
2000
Clade
1555
HPD 1458 – 1634
1879
HPD 1854-1905
1880
HPD 1862-1916
1879
HPD 1852-1894
1899
HPD 1880-1911
Clade
C4
C3
C2
C1
Other