Genomic analysis of the diversity, antimicrobial resistance and virulence potential of *Campylobacter jejuni* and *Campylobacter coli* strains from Chile

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ABSTRACT

Campylobacter jejuni and *Campylobacter coli* are the leading cause of human gastroenteritis in the industrialized world and an emerging threat in developing countries. The incidence of campylobacteriosis in South America is greatly underestimated, mostly due to the lack of adequate diagnostic methods. Accordingly, there is limited genomic and epidemiological data from this region. In the present study, we performed a genome-wide analysis of the genetic diversity, virulence, and antimicrobial resistance of the largest collection of clinical *C. jejuni* and *C. coli* strains from Chile available to date (n=81), collected in 2017-2019 in Santiago, Chile. Collection which accounts for over a third of the genome sequences available of clinical strains from South America. cgMLST analysis identified a high genetic diversity as well as 13 novel STs and alleles in both *C. jejuni* and *C. coli*. Pangenome and virulome analyses showed a differential distribution of virulence factors, including both plasmid and chromosomally encoded T6SSs and T4SSs. Resistome analysis predicted widespread resistance to fluoroquinolones, but low rates of erythromycin resistance. This study provides valuable genomic and epidemiological data and highlights the need for further genomic epidemiology studies in Chile and other South American countries to better understand molecular epidemiology and antimicrobial resistance of this emerging intestinal pathogen.

Key words: *Campylobacter jejuni*, *Campylobacter coli*, cgMLST, comparative genomics, foodborne pathogen.

48 **IMPORTANCE**

49 *Campylobacter* is the leading cause of bacterial gastroenteritis worldwide and an
50 emerging and neglected pathogen in South America. In this study, we performed an in-depth
51 analysis of the genome sequences of 69 *C. jejuni* and 12 *C. coli* clinical strains isolated from
52 Chile, which account for over a third of the sequences from clinical strains available from
53 South America. We identified a high genetic diversity among *C. jejuni* strains and the
54 unexpected identification of clade 3 *C. coli* strains, which are infrequently isolated from
55 humans in other regions of the world. Most strains harbored the virulence factors described
56 for *Campylobacter* and while ~40% of strains harbored mutation in the *gyrA* gene described
57 to confer fluoroquinolone resistance, very few strains encoded the determinants linked to
58 macrolide resistance, currently used for the treatment of campylobacteriosis. Our study
59 contributes to our knowledge of this important foodborne pathogen providing valuable data
60 from South America.

61

62 **INTRODUCTION**

63 Campylobacteriosis caused by *Campylobacter jejuni* and *Campylobacter coli* has
64 emerged as an important public health problem and is the most common bacterial cause of
65 human gastroenteritis worldwide [1,2]. It is usually associated with a self-limiting illness
66 characterized by diarrhea, nausea, abdominal cramps and bloody stools [1]. Nevertheless,
67 extraintestinal manifestations might cause long-term complications such Miller-Fisher or
68 Guillain-Barré syndrome [1]. Despite the self-limiting course, certain populations such as
69 children under 5 years of age, elderly patients, and immunocompromised patients might
70 suffer severe infections and require antimicrobial treatment [3]. In this context, the World
71 Health Organization (WHO) has included *Campylobacter* as a high priority pathogen due to
72 the worldwide emergence of strains with high level fluoroquinolone resistance [4].

73

74 In South America, there is limited data available on the prevalence of *C. jejuni* and
75 *C. coli* in comparison to other enteric bacterial pathogens [1,5,6]. In Chile, the National
76 Laboratory Surveillance Program of the Public Health Institute has reported an incidence rate
77 of campylobacteriosis of 0.1 to 0.6 cases per 100.000 persons [7]. This is low compared to
78 high-income countries, where incidence rates can reach two orders of magnitude higher [1].
79 Nevertheless, recent reports suggest that the burden of campylobacteriosis in Chile has been
80 greatly underestimated due to suboptimal diagnostic protocols [7]. Indeed, a recent report
81 suggests that *Campylobacter* spp. is emerging as the second most prevalent bacterial cause
82 of human gastroenteritis in Chile [8].

83

84 Only few reports have analyzed the genetic diversity and antimicrobial resistance
85 profiles of clinical human strains of *Campylobacter* in Chile [9–13]. Furthermore, these

86 studies have either used a limited number of strains [13] or lacked the resolution provided by
87 whole-genome sequence-based typing methods [9–12]. Therefore, larger genomic
88 epidemiology studies are needed in Chile and more broadly in South America.
89
90 We recently made available the whole-genome sequences of 69 *C. jejuni* and 12 *C.*
91 *coli* human clinical strains isolated from patients with enteric infection attending Clínica
92 Alemana in Santiago, Chile, during a 2-year period (2017-2019) [14,15]. This represents the
93 largest genome collection of clinical *Campylobacter* strains from Chile and one of the few
94 collections available from South America. In the present study, we performed a genome-
95 wide analysis of the genetic diversity, virulence potential, and antimicrobial resistance
96 profiles of these strains. Importantly, we identified a high genetic diversity of both *C. jejuni*
97 and *C. coli* strains, including 13 novel sequence types (STs) and alleles. Resistome analysis
98 predicted a widespread resistance to fluoroquinolones, but decreased resistance to
99 tetracycline in comparison to the reported resistance from other regions of the world, in
100 addition to the differential distribution of virulence factors, including plasmids and
101 chromosomally encoded T6SS and T4SS.
102

103 **MATERIALS AND METHODS**

104 *Genome data set, sequencing and reannotation*

105 The genome sequence dataset analyzed in this study is listed in **Table S1** and includes our
106 recently published collection of human clinical strains of *C. jejuni* and *C. coli* from Chile
107 [14,15]. We additionally included the draft whole-genome sequence of *C. jejuni* strain
108 CFSAN096303, from the same collection of isolates. CFSAN096303 was sequenced as
109 described in [14], using the NextSeq sequencer (Illumina) obtaining an estimated genome
110 average coverage of 90X. The genome was assembled using the CLC Genomics Workbench
111 v9.5.2 (Qiagen) following the previously used pipeline [14] and was deposited under
112 accession number WXZQ01000000. Fasta files of all draft genomes were reordered to the
113 reference genomes of the *C. coli* aerotolerant strain OR12 (accID GCF_002024185.1) and
114 *C. jejuni* strain NCTC 11168 (accID GCF_000009085.1) using the Mauve Contig Mover
115 (MCM) from the Mauve package [16]. The ordered genomes were reannotated using Prokka
116 [17] with the same reference files mentioned above and by forcing GenBank compliance.
117 Draft genome sequence reannotations and sequence fasta files are available as a
118 supplementary dataset on Zenodo https://doi.org/10.5281/zenodo.3925206.

119

120 *MLST and core genome MLST (cgMLST) analysis:*

121 Genome assemblies were mapped against an MLST scheme based on seven housekeeping
122 genes [18] and the 1,343-locus cgMLST scheme [19], using Ridom SeqSphere software
123 (Münster, Germany) [20]. *C. hepaticus HV10* (accID GCF-001687475.2), *C. upsaliensis*
124 *DSM 5365* (accID GCF-000620965.1), *C. lari RM2100* (accID GCF-000019205.1), *C. jejuni*
125 *NCTC 11168* (accID GCF-000009085.1), *C. jejuni doylei 269.97* (accID GCA-
126 000017485.1), aerotolerant *C. coli OR12* (accID GCF-002024185.1), *C. coli BIGS0008*

127 (accID GCA-000314165.1), *C. coli 76339* (accID GCA-000470055.1), and *C. coli*
128 *H055260513* (accID GCA-001491555.1) were used as reference genomes. Phylogenetic
129 comparison of the 81 *Campylobacter* genome sequences was performed using a neighbor-
130 joining tree based on a distance matrix of the core genomes of all strains. STs and CCs were
131 determined after automated allele submission to the *Campylobacter* PubMLST server [18].
132 Comparative cgMLST analysis with *C. jejuni* and *C. coli* clinical strains worldwide was
133 performed with genomes available at the NCBI database. Annotations and visualizations
134 were performed using iTOL v.4 [21].
135
136 *Virulome, resistome and comparative genomic analysis*
137 A local BLAST database was constructed using fasta files of all sequenced genomes and the
138 makeblastdb program from BLAST (NCBI) [22], which was screened for the nucleotide
139 sequence of known pathogenicity genes using BLASTn (version 2.8.1) [22]. A 90% sequence
140 length and 90% identity threshold were used to select positive matches. The fasta file
141 containing the nucleotide sequence of virulence genes screened can be found at
142 https://doi.org/10.5281/zenodo.3925206. The C257T mutation in *gyrA* and mutations
143 A2058C and A2059G in the genes for 23S RNA were searched manually by BLAST and
144 sequences were aligned to the reference gene from strain *Campylobacter jejuni* ATCC 33560
145 using ClustalX [23]. Antimicrobial resistance genes were screened in each *Campylobacter*
146 genome using the ABRicate pipeline [24], using the Resfinder [25], CARD [26], ARG-
147 ANNOT [27] and NCBI ARRGD [28] databases. BLAST-based genome comparisons and
148 visualization of genetic clusters were performed using EasyFig [29].
149
150

151 *Pangenome and plasmid screening analysis*

152 Annotated genome sequences were compared using Roary [30], setting a minimum
153 percentage identity of 90% for BLASTp. Pangenome visualization was performed using
154 roary_plots.py. Additionally, the file gene_presence_absence.csv produced by Roary was
155 analyzed using a spreadsheet program to find genes that are shared by subgroups of isolates.
156 Venn diagrams were constructed using the tool available at
157 http://bioinformatics.psb.ugent.be/webtools/Venn/. Plasmids screening was performed on
158 FASTQ files using PlasmidSeeker [31], based on the default bacterial plasmid database. The
159 inhouse database of known plasmids from *C. coli* or *C. jejuni* and the output table for the
160 presence/abscence of gene content in the Roary analysis can be found in
161 https://doi.org/10.5281/zenodo.3925206. Pangenome BLAST analysis was performed using
162 GView (https://server.gview.ca/) [32].

163

164 **RESULTS**

165

166 *Phylogenetic analysis of clinical Campylobacter strains.*

167

168 cgMLST analysis showed high genetic diversity among the 69 *C. jejuni* strains, as
169 shown by the 14 CCs and 31 distinct STs identified (**Figure 1A**). Almost 93% (n=64) of the
170 *C. jejuni* strains belonged to one of the CCs previously described for this species. Among our
171 strains, CC-21 was the most common (37.6%) and diverse (5 distinct STs) (**Figure 1A, Table**
172 **S1**). ST-1359 was the most prevalent ST of CC-21 (48%) of all *C. jejuni* strains in this study
173 (17.4%) (**Figure 1A and Table S1**). In addition to CC-21, we identified high prevalence
174 rates of CC-48 (13%) and CC-52 (7.3%). ST-475 from CC-48 was the second most prevalent
175 ST (11.6%) identified (**Figure 1A**).

176

177 cgMLST analysis of the 12 *C. coli* strains from our collection revealed that 83%
178 (10/12) of the strains belonged to clade 1, while 17% (2/12) belonged to clade 3 and none to
179 clade 2. Most clade 1 strains (8/10) belonged to CC-828 and included four previously
180 described STs (828, 829, 1173, and 1556) and one novel ST (10201). Only one strain from
181 clade 1 belonged to the usually common CC-1150 (strain CFSAN093253 from ST-10203),
182 and another strain could not be assigned to any clonal complex (strain CFSAN096322 from
183 ST-10202). Both clade 3 strains belonged to ST-10204.

184

185 A global cgMLST analysis comparing our 81 *Campylobacter* genomes with other *C.*
186 *jejuni* and *C. coli* genomes available from the NCBI database was performed. The strains of
187 our collections from Central Chile showed great diversity and did not group with any existing

188 clusters (**Figure 1B).** The reconstructed phylogenetic trees and associated metadata of
189 clinical strains from Chile in the global context of *C. jejuni* and *C. coli* sequenced strains can
190 be interactively visualized on Microreact at https://microreact.org/project/VbEQsZtQD.
191
192 *Pangenome analysis of Campylobacter strains.*
193
194 To identify the genetic elements that are shared and distinct among the Chilean *C.*
195 *jejuni* and *C. coli* strains, we performed pangenome analysis of the 81 strains from both
196 species (**Figure 2**). The analysis identified a pangenome of 6609 genes for both species and
197 identified the core genes shared between *C. jejuni* and *C. coli* (532 genes) and the core
198 genome of each species (1300 genes for *C. jejuni* and 1207 genes for *C. coli*). In addition,
199 the analysis was able to identify the differences among the core and accessory genomes of
200 *C. coli* strains from clade 1 and clade 3. *C. coli* strains from clade 1 harbored 340 core genes
201 absent from clade 3 core genes, which in turn, harbored 159 core genes not present in clade
202 1 core genes (**Figure 2**). The estimated core genome for *C. jejuni* in our study (1300 genes)
203 was close to the 1343 core genes identified in the development of the cgMLST scheme of *C.*
204 *jejuni* [19] and in agreement with recent reports [33] (**Table S2**). In addition, a significant
205 variation in the accessory genome between *C. jejuni* and *C. coli* strains was identified,
206 confirming the high genetic variability of *Campylobacter*. Accessory genes that are common
207 to a particular cluster seem to contribute to modifications of carbohydrates and flagella as
208 well as antibiotic resistance. There is also a proportion of genomes harboring phage
209 sequences as well as T4SS and T6SS-related genes (see below).
210
211 *Genomic analysis of the resistome.*

212

213 To determine the resistome, we performed *in silico* analysis to identify genes
214 associated with antimicrobial resistance (**Figure 3**) by screening each *Campylobacter*
215 genome by means of the ABRicate pipeline [24] using the Resfinder, CARD, ARG-ANNOT
216 and NCBI ARRGD databases. We additionally performed sequence alignments to identify
217 point mutations in the *gyrA* and 23S rRNA genes. The *cmeABC* operon was present in all *C.*
218 *jejuni* and *C. coli* strains analyzed. This operon encodes a common multidrug efflux pump
219 characterized in different *Campylobacter* species, which mainly confers resistance to
220 fluoroquinolones and in some cases to macrolides [34]. Additionally, the point mutation in
221 the quinolone resistance-determining region (QRDR) of the *gyrA* gene, leading to the T86I
222 substitution in GyrA, was identified in 54.3% of the strains. This mutation confers high levels
223 of resistance to fluoroquinolones [35].

224

225 In contrast, a much lower fraction of the strains harbored resistance markers for other
226 antimicrobials. 22.2% of the strains harbored the *tetO* gene, which confers tetracycline
227 resistance in *Campylobacter* spp [36]. Furthermore, we did not detect neither mutation of the
228 23S rRNA gene nor presence of the *ermB* gene, both associated to macrolide resistance [37].
229 Finally, we also found a high proportion of strains (79%) harboring the blaOXA-61 gene
230 among *C. jejuni* and *C. coli* strains. This genetic marker is associated with β-lactam resistance
231 and has high prevalence in *Campylobacter* strains worldwide [38].

232

233 *Presence and diversity of virulence gene content among Chilean Campylobacter strains.*

234

235 To determine the virulence gene content of the *C. jejuni* and *C. coli* strains, we first
236 assembled a list of 220 potential virulence genes, including genes described in the Virulence
237 Factor Database [39] and genes reported to contribute to the virulence of *Campylobacter* in
238 the literature [40–43]. These genes were grouped into five distinct categories (adhesion and
239 colonization, invasion, motility, secretion systems and toxins) and were used to screen each
240 of the 81 genomes using BLASTn (**Figure 4**, **Figure S1** and **Table S3** for the full list). Most
241 of the described factors involved in adhesion and colonization (*cadF*, *racR*, *jlpA, pdlA, dnaJ,*
242 *aphC*) were prevalent just among the *C. jejuni* strains and were not associated with any
243 particular STs (**Figure 4**, **Table S3**).
244
245 The genes responsible for the production of the CDT toxin (*cdtA*, *cdtB* and *cdtC*) were
246 also found in most strains of *C. jejuni* and in every *C. coli* strain tested. The *cdtA* and *cdtB*
247 genes were not detected in the *C. jejuni* strain CFSAN093229. In contrast, the *ciaB* gene was
248 identified only in *C. jejuni* (in 98.7% of the strains) (**Figure 4**). The product of this gene is
249 involved in cecal colonization in chickens and in translocation into host cells in *C. jejuni*
250 [44,45]. The absence of this gene in *C. coli* might suggest that it does not play a crucial role
251 in the pathogenesis of this *Campylobacter* species.
252
253 The major differences among *C. jejuni* strains were observed for genes located at the
254 *cps* locus (Cj1413c -Cj1442) involved in capsule polysaccharide synthesis, the LOS locus
255 (C8J1074-C8J1095) involved in lipooligosaccharide synthesis, a small set of genes in the O-
256 linked flagellin glycosylation island (Cj1321-Cj1326) and the T6SS and T4SS gene clusters
257 (**Figure 4**, **Figure S1 and Table S3**). Additionally, *C. coli* strains carried most of the genes
258 known to be associated with invasiveness in *C. jejuni*, including the *iamb, flaC* and *ciaB*

259 genes. Both *C. coli* clades also harbored some genes associated with other steps of infection
260 such as the *cdt* toxin gene and most of the flagellar biosynthesis and chemiotaxis-related
261 genes described for *C. jejuni* (**Figure S1**). its relevance in pathogenicity remains unclear [46].
262
263 *Distribution of the T6SS and T4SS gene clusters.*
264
265 Our analysis identified the T6SS gene cluster in 9 *C. jejuni* strains and in 1 *C. coli*
266 strain from clade 1 (**Figure 4**). As shown in **Figure 5A**, whenever present the genetic
267 structure and the sequence identity of this cluster was highly conserved among *C. jejuni* and
268 *C. coli* strains. For strains with genomes that were not closed, we were not able to confirm
269 whether the *tssI* gene is located within the genetic context of the of the T6SS cluster, since it
270 was not assembled in the same contig as the rest of the cluster. The *Campylobacter* T6SS
271 gene cluster is often encoded within the CjIE3 conjugative element in the chromosome of *C.*
272 *jejuni* [41], but it can also be found in plasmids [47,48]. Since most of our sequenced strains
273 correspond to draft genome assemblies, we could not determine whether the T6SSs were
274 plasmid or chromosomally encoded in most of them. However, we have closed the genomes
275 of 17 of these strains [15]. In two of these strains (CFSAN093238 and CFSAN093227), T6SS
276 was inserted in the chromosomal CjIE3 element, while in strain CFSAN093246, it was
277 plasmid-encoded. Sequence-based analysis of this plasmid showed a high degree of identity
278 with plasmid pMTVDDCj13-2 (**Figure 6A**), which encodes a complete T6SS gene cluster
279 [48].
280
281 Genome analysis identified a T4SS gene cluster in 12 strains of *C. jejuni* and 1 strain
282 of *C. coli* clade 1 (**Figure 4**). It harbored the 16 core genes and was correctly assembled in

283 individual contigs in 11 strains, which allowed the analysis of the genetic context of the
284 cluster. In each strain, the T4SS cluster had a conserved genetic structure (**Figure 5B**). One
285 of the main differences between the T4SS gene clusters encoded in plasmids pTet and pCC31
286 is the sequence divergence of the *cpp33* gene [49]. While *cp33* present in strain
287 CFSAN096321 showed high identity with the gene harbored in the pTet plasmid, every other
288 strain possessed high identity to the *cp33* encoded in pCC31 plasmid. Both T4SS have been
289 involved in bacterial conjugation and are not directly linked to the virulence of *C. jejuni* and
290 *C. coli* strains [49]. As shown in **Figure 6B**, plasmids from closed genome strains
291 CFSAN093226 and CFSAN096297 share significant similarity with both the pCC31 and
292 pTET plasmids beyond the T4SS gene cluster. For all other strains, BLASTn analysis of each
293 of our draft genome assemblies identified most of the plasmid genes found in
294 pCFSAN093226, pCFSAN096297 and pTet-like plasmids, suggesting that all the T4SS gene
295 clusters identified in these strains are encoded in plasmids as well.

296 **DISCUSSION**

297

298 In South America, Campylobacteriosis is an emerging and neglected foodborne
299 disease. Here, we performed an in-depth genome analysis of the largest collection of clinical
300 *Campylobacter* strains from Chile, which accounts for over 35% of the genome sequences
301 available of clinical strains from South America.

302

303 cgMLST analysis provided insight into key similarities and differences in comparison
304 to the genetic diversity reported for clinical *Campylobacter* strains worldwide. *C. jejuni*
305 strains were highly diverse being the CC-21 the most common. CC-21 is the largest and most
306 widely distributed CC, representing 18.9% of all *C. jejuni* strains submitted to the PubMLST
307 database. The high prevalence of ST-1359 in our study was unexpected, since it is infrequent
308 within the PubMLST database (0.06%, February 2020). However, ST-1359 has been
309 recognized as a prominent ST in Israel [50]. ST-45, the second most prevalent ST worldwide
310 (4.2%), was not identified among our strains. Previous reports from Chile identified four
311 major CCs, including CC-21 (ST-50), CC-48 (ST-475), CC-257 (ST-257), and CC-353 (ST-
312 353) [9,13]. Our study is consistent with these reports, except for the high prevalence of ST-
313 1359. Further studies are needed to determine if ST-1359 represents an emergent ST. Our
314 study also provided the first genome data of clinical *C. coli* strains from Chile. *C. coli* is
315 divided into three genetic clades [51]. Clade 1 strains are often isolated from farm animals
316 and human gastroenteritis cases while clade 2 and 3 strains are mainly isolated from
317 environmental sources [51]. We showed that clade 1 strains from CC-828 were most
318 prevalent, which is consistent with the worldwide distribution of this CC [51]. Interestingly,
319 despite the lower amount of *C. coli* strains isolated in the 2-year period of our study (n=12),

320 we were able to identify strains from the uncommon clade 3. Therefore, larger genomic
321 epidemiological studies are needed to determine the prevalence of clade 3 strains in Chile
322 and South America.
323
324 Although most *Campylobacter* infections are self-limiting, antibiotics are indicated
325 for patients with persistent and severe gastroenteritis, extraintestinal infections, or who are
326 immunocompromised [1]. The high percentage of *C. jejuni* strains with a mutation of the
327 *gyrA* gene, which confers quinolone resistance, is consistent with recent studies from Chile,
328 reporting ciprofloxacin resistance of 30-60% [9,11–13]. One study reported that most
329 resistant strains were associated with ST-48 [52], but we did not find an association to any
330 particular STs. However, all of the strains belonging to ST-1359, the most frequent ST
331 identified, harbored this mutation. Since fluoroquinolones are not the first choice
332 antimicrobials for human campylobacteriosis, it has been suggested that high levels of
333 resistance might be a consequence of their broad use in animal husbandry in Latin America
334 [53]. These animal production practices might be responsible for the dissemination of
335 antibiotic resistance genes among Chilean *Campylobacter* strains.
336
337 None of the clinical strains harbored the mutation of the 23S rRNA gene nor presence
338 of the *ermB* gene which confers macrolide resistance, which is consistent with their choice
339 as first line treatment for *Campylobacter* infections [37]. Tetracycline resistance is
340 widespread among *Campylobacter* strains worldwide [35]. Recently, the European Union
341 reported high levels of resistance in *C. jejuni* strains (45.4%) and even higher levels in *C.*
342 *coli* (68.3%). Tetracycline is especially used in poultry industry worldwide [54], and might
343 serve as an important reservoir for resistant strains [55]. Indeed, it was recently reported that

344 32.3% of *C. jejuni* strains isolated from poultry in Chile were resistant to tetracycline [12].
345 Our data are consistent with this report, as 22% of the clinical *C. jejuni* strains were resistant.
346 This potential emergence of resistance highlights the need for a permanent surveillance
347 program to implement control measures for tetracycline usage in animal production.
348
349 While the ability of *Campylobacter* to cause human disease is thought to be
350 multifactorial, there are several genes associated with the virulence of *Campylobacter*, even
351 though their role in campylobacteriosis is not fully understood [56]. Genome analysis showed
352 that clinical *C. jejuni* strains harbored most of the known virulence factors described for the
353 species. On the contrary, *C. coli* strains lacked most virulence genes described for *C. jejuni*
354 (**Figure 4**). Since most of our knowledge regarding the virulence of *Campylobacter* comes
355 from studies performed in *C. jejuni*, this highlights the need for a better understanding of
356 how *C. coli* strains cause disease.
357
358 To date, there is only one T6SS cluster described in *Campylobacter* [40]. This T6SS
359 has been shown to contribute to host cell adherence, invasion, resistance to bile salts and
360 oxidative stress [57–59], and required for the colonization of murine [57] and avian [59]
361 infection models. We identified a complete T6SS gene cluster in 9 *C. jejuni* and in 1 *C. coli*
362 strain (**Figure 4**). No correlation was found between presence of the T6SS and any particular
363 ST, which differs from what has been described in Israel, where most T6SS-positive strains
364 belong to ST-1359 [50]. Although ST-1359 was the most common ST described in this study,
365 none of the strains carried a T6SS.
366

367 Two distinct T4SSs have been described in *Campylobacter*. One T4SS is encoded in
368 the pVir plasmid of *C. jejuni* and has been shown to contribute to the invasion of INT407
369 cells and the ability to induce diarrhea in a ferret infection model [42,60]. The second T4SS
370 is encoded in the pTet and pCC31 plasmids of *C. jejuni* and *C. coli,* which contributes to
371 bacterial conjugation and is not directly linked to virulence [49]. Each of the T4SS gene
372 clusters identified in our strains showed a high degree of identity to the pTet and pCC31
373 T4SSs. Suggesting that they do not directly contribute to virulence, but they could facilitate
374 horizontal gene transfer events that could lead to increase fitness and virulence.
375
376 Altogether, we provide valuable epidemiological and genomic data of the diversity,
377 virulence and resistance profiles of a large collection of clinical *Campylobacter* strains from
378 Chile. Further studies are needed to determine the dynamics of transmission of pathogenic
379 *Campylobacter* to humans and the potential emergence of new virulence and antimicrobial
380 resistance markers in order to provide actionable public health data to support the design of
381 strengthened surveillance programs in Chile and South America.
382

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523

524 **FIGURES**

525

526

527

528

529

530 **Figure 1.** Phylogenetic analysis of clinical *C. jejuni* and *C. coli* strains from Chile. A)
531 Phylogenetic tree is based on cgMLST performed with RIDOM SeqSphere^{+} and visualized
532 by iTOL. STs are shown in colored boxes for each strain, and tree branches are color coded
533 to highlight *C. jejuni* and *C. coli* strains from clades 1, 2 and 3. **B)** Global cgMLST analysis
534 of clinical *C. jejuni* and *C. coli* strains. Strains from this study are highlighted in red.

535

536 **Figure 2.** Visualization of the pangenome of the *C. jejuni* and *C. coli* strains. Pangenome
537 analysis was performed using Roary. Core and accessory genomes are highlighted with red
538 and black brackets, respectively. cgMLST phylogenetic tree of Figure 1A is shown on the
539 left. A Venn diagram highlighting the number of core genes present in *C. jejuni* and *C. coli*
540 strains from clades 1 and 3 is shown on the right.

541

542 **Figure 3.** Distribution of antimicrobial resistance genes. Binary heatmaps show the presence
543 and absence of antimicrobial resistance genes. Colored cells represent the presence of genes.
544 cgMLST phylogenetic tree of Figure 1A is shown on the left. Strain names are color coded

545 to highlight phylogenetic groups: *C. jejuni* strains (light green), *C. coli* clade 1 strains
546 (orange) and *C. coli* clade 3 strains (magenta).

547

548 **Figure 4.** Distribution of virulence-related genes. Binary heatmaps show the presence and
549 absence of virulence genes. Colored cells represent the presence of genes. cgMLST
550 phylogenetic tree of Figure 1A is shown on the left. Strain names are color coded to highlight
551 *C. jejuni* strains (green), *C. coli* clade 1 strains (orange) and *C. coli* clade 3 strains (purple).

552

553 **Figure 5.** Comparative genomic analysis of T6SS and T4SS gene clusters. **A)** T6SS gene
554 clusters of *C. jejuni* and *C. coli* strains (bold) compared to the cluster of *C. jejuni* strain 108.
555 Genes *tagH*, *tssD*, *tssB* and *tssC* are shown in color. **B)** T4SS gene clusters of *C. jejuni* strains
556 in comparison to the T4SS gene clusters of pTet and pCC31. The *cp33* gene is highlighted
557 in orange. BLASTn alignments were performed and visualized using EasyFig.

558

559 **Figure 6.** Comparative genomic analysis of plasmids encoding T6SS **(A)** and T4SS **(B)** gene
560 clusters. Pangenome BLAST analysis was performed using the Gview server. T6SS and
561 T4SS gene clusters are highlighted in red brackets.

562 **Figure S1.** Distribution of the presence (red) and absence (blue) of virulence-related genes
563 in *C. jejuni* and *C. coli* strains from this study.

564

565 **Table S1**. Metadata of strains used in this study.

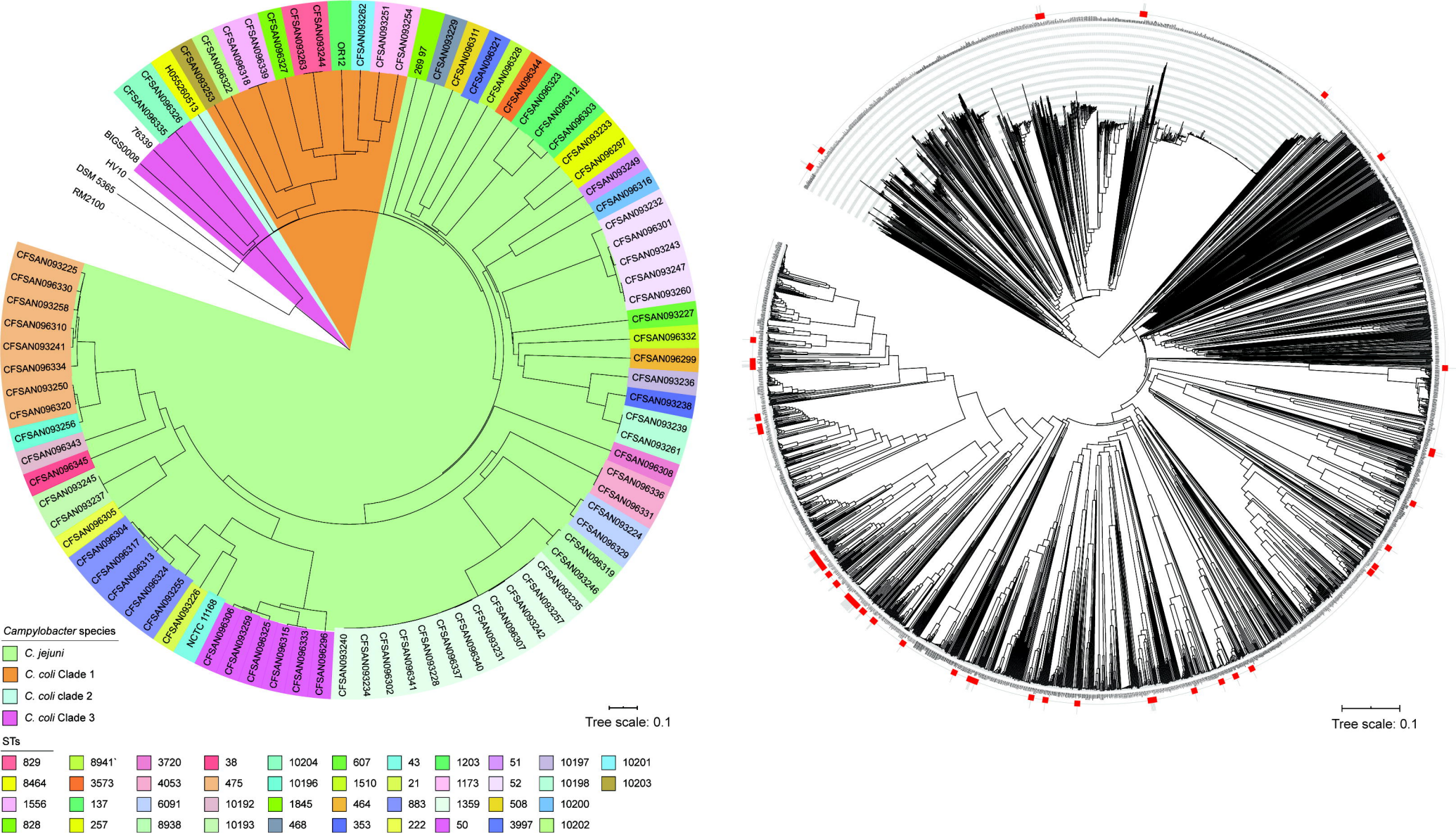
566

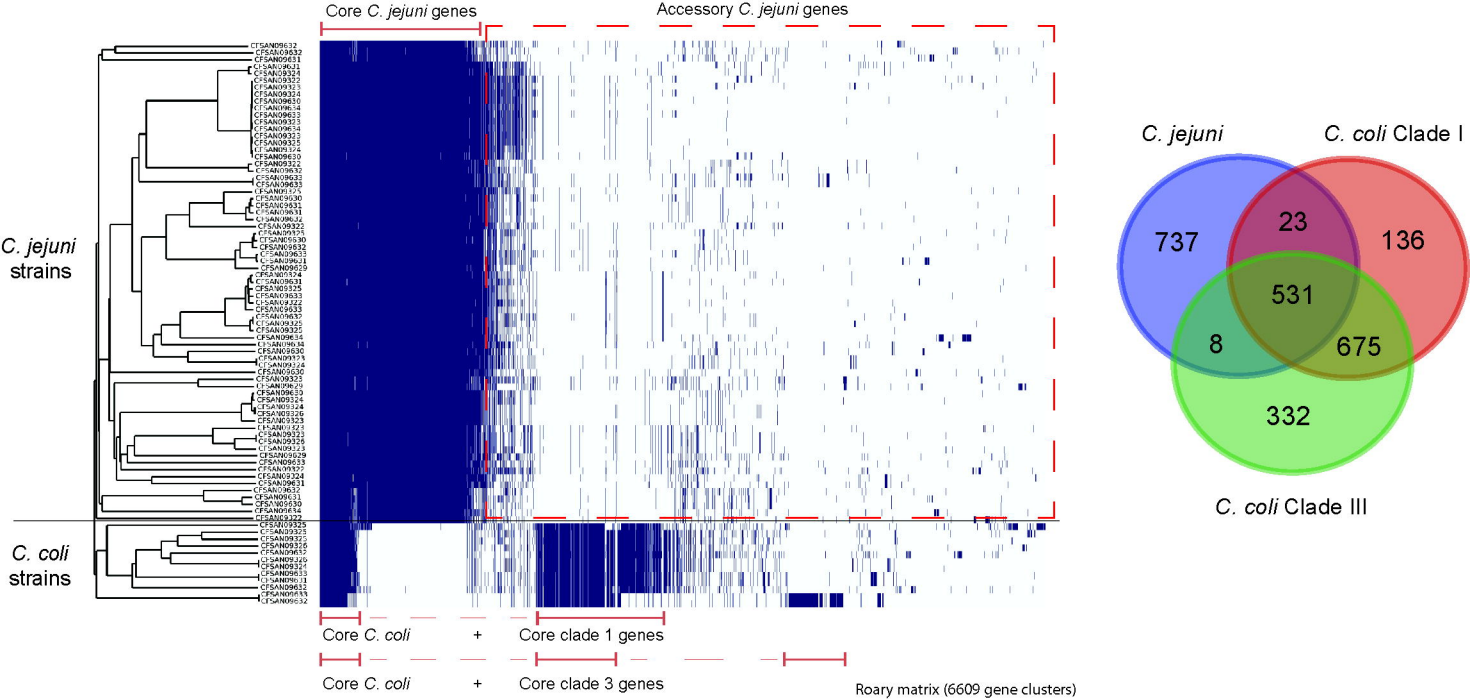
567 **Table S2.** Frequency and distribution of CCs and STs of strains of this study.

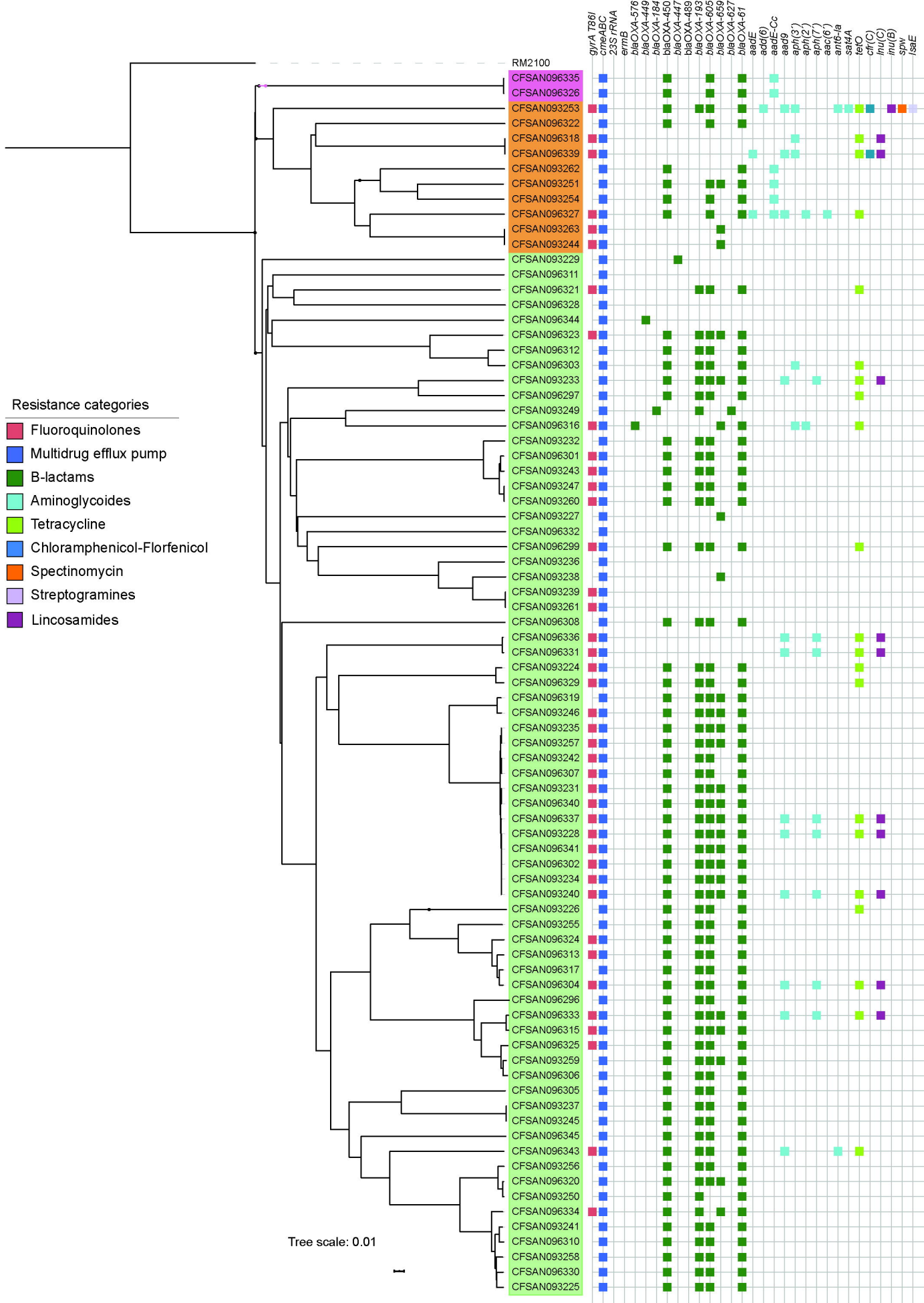
568

569 **Table S3.** Identification of virulence genes. Cells with a red background represent

570 presence. Empty cells represent absence of the gene in each genome.


Campylobacter species
C. jejuni
C. coli Clade 1
C. coli clade 2
C. coli Clade 3
Tree scale: 0.1
STs
829
8941`
3720
38
10204
607
43
1203
51
10197
10201
8464
3573
4053
475
10196
1510
21
1173
52
10198
10203
1556
137
6091
10192
1845
464
883
1359
508
10200
828
257
8938
10193
468
353
222
50
3997
10202
Tree scale: 0.1


Core C. jejuni genes
Accessory C. jejuni genes
C. jejuni strains
C. coli strains
Core C. coli + Core clade 1 genes
Core C. coli + Core clade 3 genes
Roary matrix (6609 gene clusters)
C. jejuni
C. coli Clade I
C. coli Clade III
737
23
136
531
8
675
332


gyrA T86I
cmeABC
23S rRNA
ermB
blaOXA-576
blaOXA-449
blaOXA-184
blaOXA-450
blaOXA-447
blaOXA-489
blaOXA-193
blaOXA-605
blaOXA-659
blaOXA-627
blaOXA-61
aadE
add(6)
aadE-Cc
aad9
aph(3')
aph(2')
aph(7')
aac(6')
ant6-Ia
sat4A
tetO
cfr(C)
lnu(C)
lnu(B)
spw
lsaE
RM2100
CFSAN096335
CFSAN096326
CFSAN093253
CFSAN096322
CFSAN096318
CFSAN096339
CFSAN093262
CFSAN093251
CFSAN093254
CFSAN096327
CFSAN093263
CFSAN093244
CFSAN093229
CFSAN096311
CFSAN096321
CFSAN096328
CFSAN096344
CFSAN096323
CFSAN096312
CFSAN096303
CFSAN093233
CFSAN096297
CFSAN093249
CFSAN096316
CFSAN093232
CFSAN096301
CFSAN093243
CFSAN093247
CFSAN093260
CFSAN093227
CFSAN096332
CFSAN096299
CFSAN093236
CFSAN093238
CFSAN093239
CFSAN093261
CFSAN096308
CFSAN096336
CFSAN096331
CFSAN093224
CFSAN096329
CFSAN096319
CFSAN093246
CFSAN093235
CFSAN093257
CFSAN093242
CFSAN096307
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CFSAN096340
CFSAN096337
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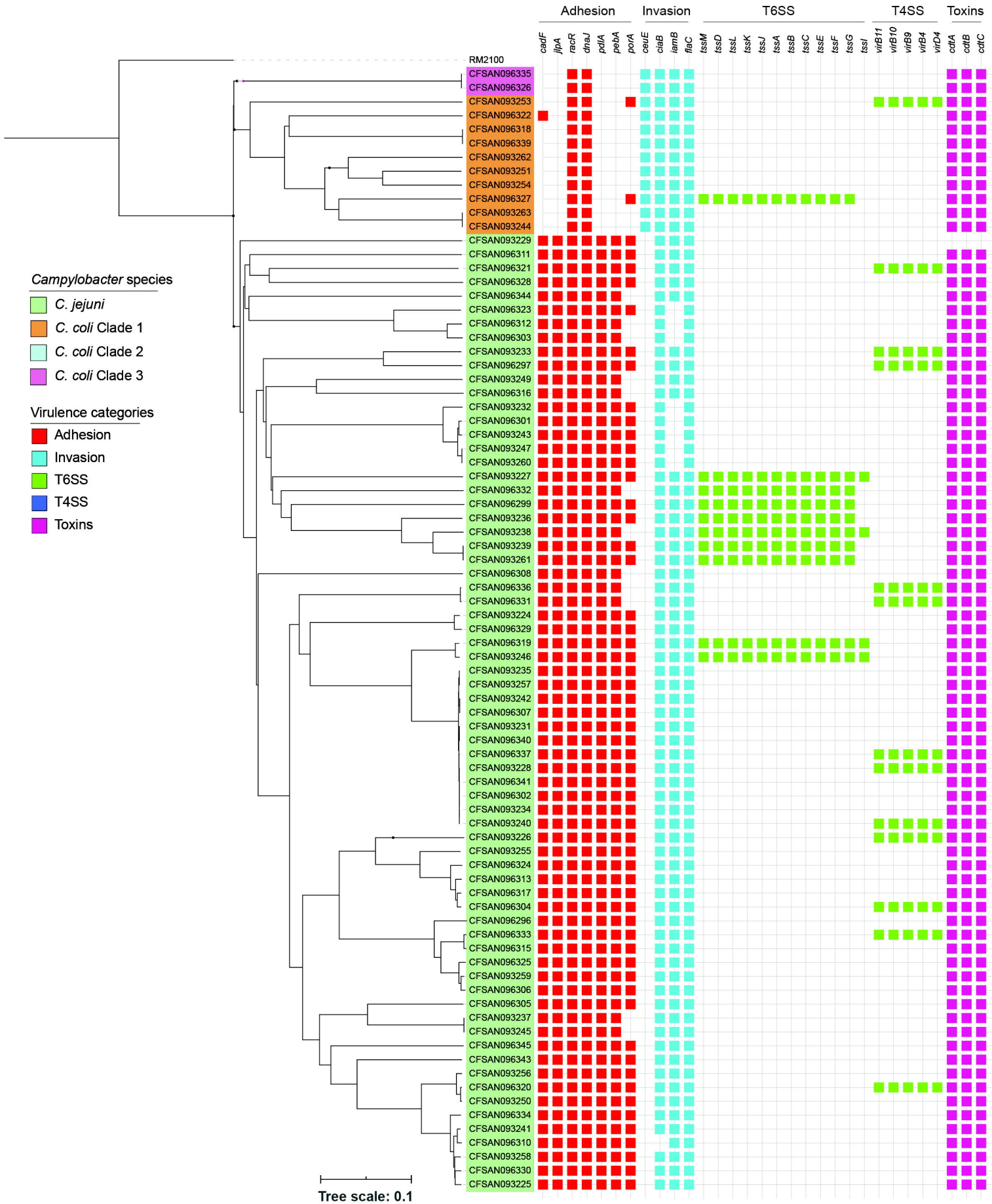
CFSAN096313
CFSAN096317
CFSAN096304
CFSAN096296
CFSAN096333
CFSAN096315
CFSAN096325
CFSAN093259
CFSAN096306
CFSAN096305
CFSAN093237
CFSAN093245
CFSAN096345
CFSAN096343
CFSAN093256
CFSAN096320
CFSAN093250
CFSAN096334
CFSAN093241
CFSAN096310
CFSAN093258
CFSAN096330
CFSAN093225
Resistance categories
Fluoroquinolones
Multidrug efflux pump
B-lactams
Aminoglycoides
Tetracycline
Chloramphenicol-Florfenicol
Spectinomycin
Streptogramines
Lincosamides
Tree scale: 0.01


Adhesion
Invasion
T6SS
T4SS
Toxins
cadF
jlpA
racR
dnaJ
pdlA
pebA
porA
ceuE
claB
iamB
flaC
tssM
tssD
tssL
tssK
tssJ
tssA
tssB
tssC
tssE
tssF
tssG
tssI
virB11
virB10
virB9
virB4
virD4
cdtA
cdtB
cdtC
RM2100
CFSAN096335
CFSAN096326
CFSAN093253
CFSAN096322
CFSAN096318
CFSAN096339
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CFSAN093251
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CFSAN093233
CFSAN096297
CFSAN093249
CFSAN096316
CFSAN093232
CFSAN096301
CFSAN093243
CFSAN093247
CFSAN093260
CFSAN093227
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CFSAN093237
CFSAN093245
CFSAN096345
CFSAN096343
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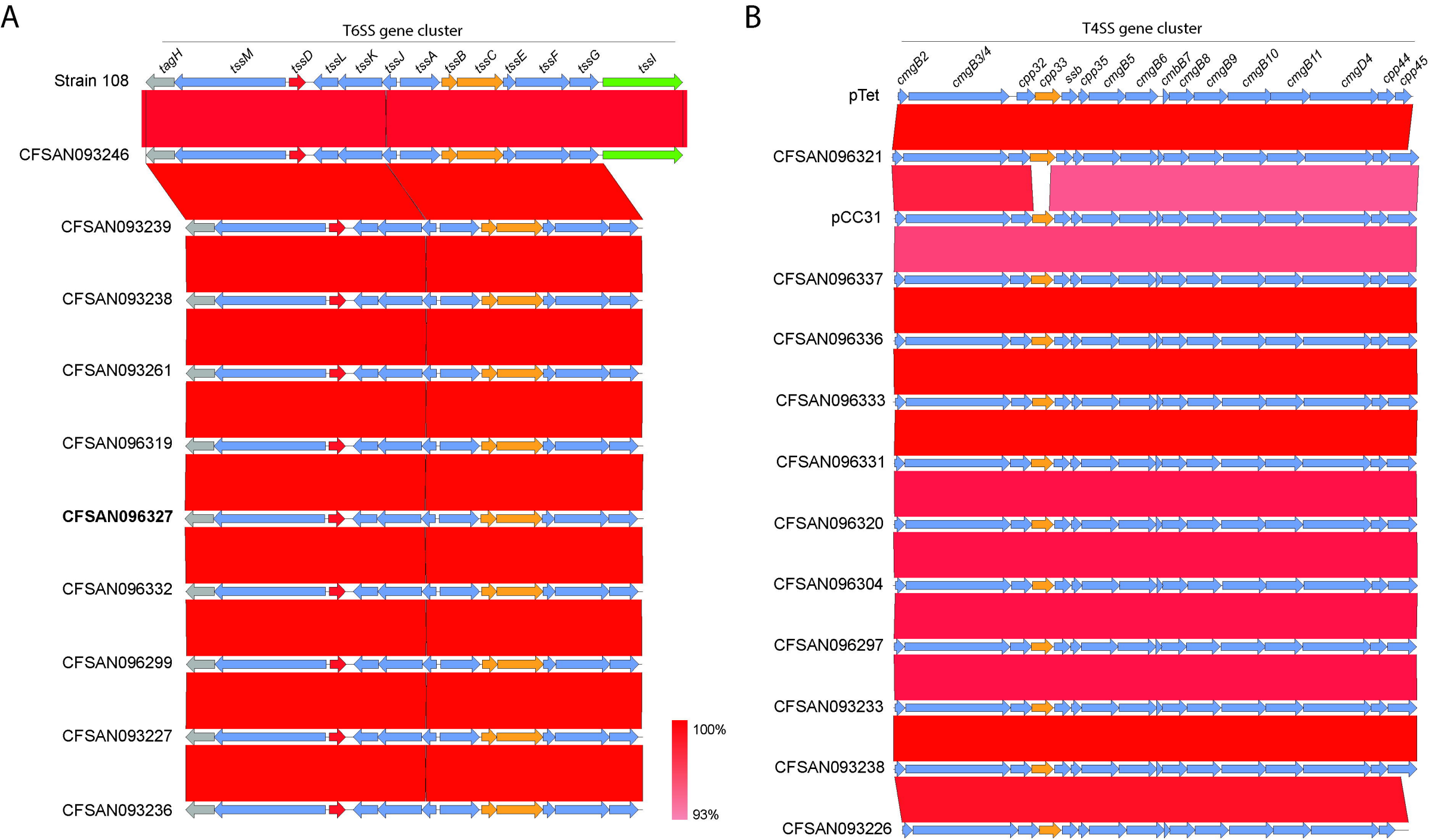
CFSAN096320
CFSAN093250
CFSAN096334
CFSAN093241
CFSAN096310
CFSAN093258
CFSAN096330
CFSAN093225
Campylobacter species
C. jejuni
C. coli Clade 1
C. coli Clade 2
C. coli Clade 3
Virulence categories
Adhesion
Invasion
T6SS
T4SS
Toxins
Tree scale: 0.1


A
T6SS gene cluster
tagH
tssM
tssD
tssL
tssK
tssJ
tssA
tssB
tssC
tssE
tssF
tssG
tssI
Strain 108
CFSAN093246
CFSAN093239
CFSAN093238
CFSAN093261
CFSAN096319
CFSAN096327
CFSAN096332
CFSAN096299
CFSAN093227
CFSAN093236
100%
93%
B
T4SS gene cluster
cmgB2
cmgB3/4
cpp32
cpp33
ssb
cpp35
cmgB5
cmgB6
cmbB7
cmgB8
cmgB9
cmgB10
cmgB11
cmgD4
cpp44
cpp45
pTet
CFSAN096321
pCC31
CFSAN096337
CFSAN096336
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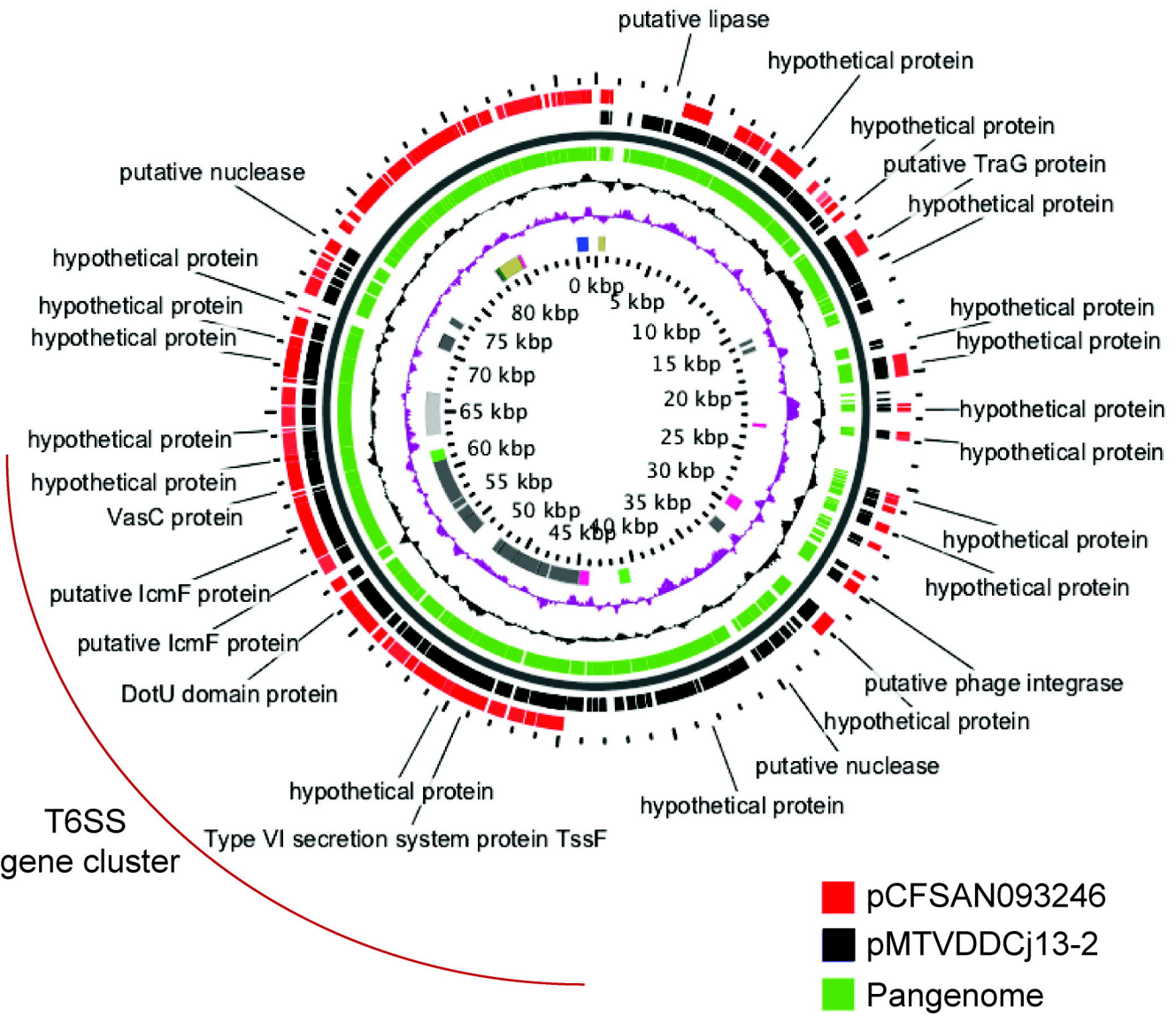
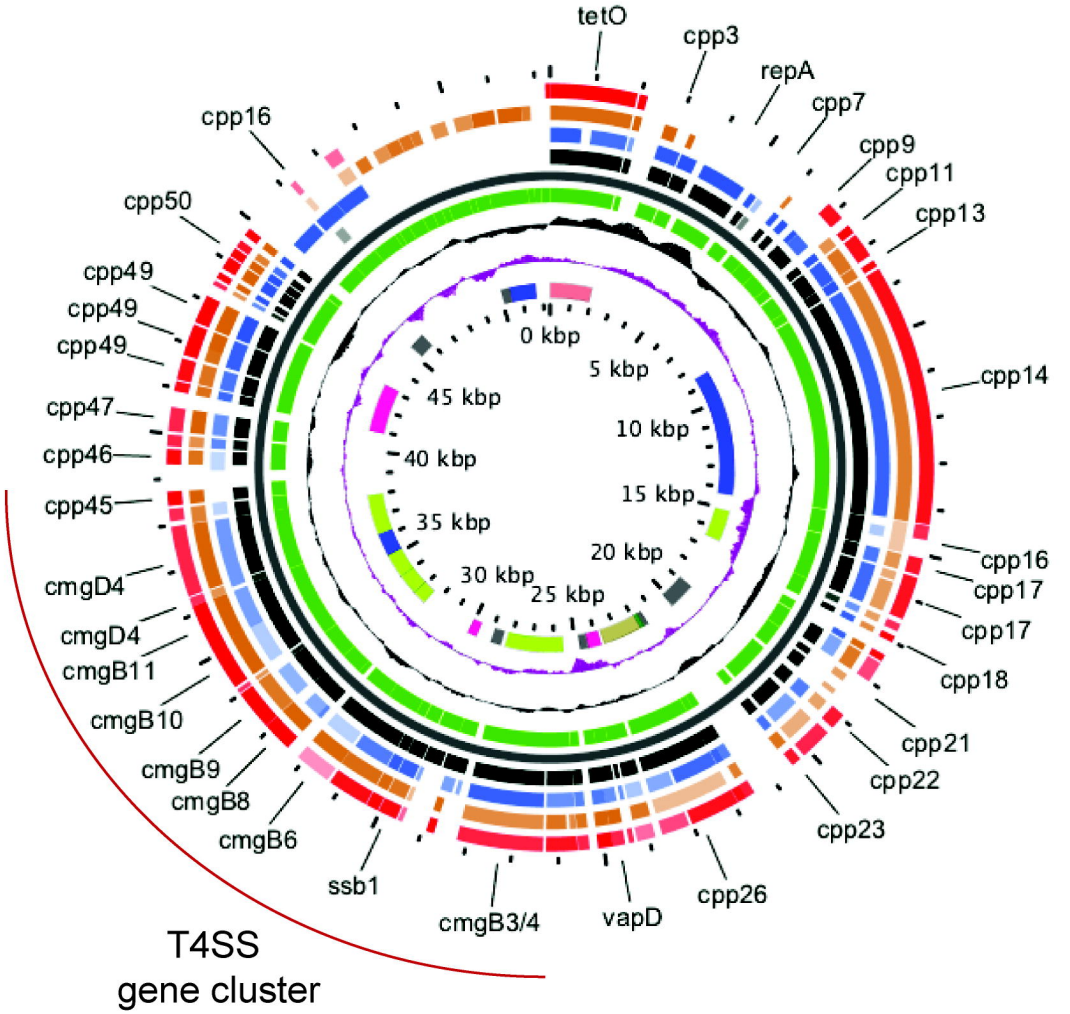


T6SS
gene cluster



T4SS
gene cluster

pCFSAN093246
pMTVDDCj13-2
Pangenome

pCFSAN093226
pCFSAN096297
pCC31
pTet
Pangenome