

1 **Local accessory gene sharing drives lineage-specific acquisition of**
2 **antimicrobial resistance in Egyptian *Campylobacter* spp.**

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25 Archive, associated with BioProject PRJNA576513
26 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576513>).

27 **Abstract**

28 *Campylobacter* is the most common cause of bacterial gastroenteritis worldwide and diarrheal disease
29 is a major cause of child morbidity, growth faltering and mortality in low- and middle-income
30 countries (LMICs). Despite evidence of high incidence and differences in disease epidemiology, there
31 is limited genomic data from studies in developing countries. In this study, we characterised the
32 genetic diversity and accessory genome content of a collection of *Campylobacter* isolates from Cairo,
33 Egypt. In total, 112 *Campylobacter* isolates were collected from broiler carcasses (n=31), milk and
34 dairy products (n=24) and patients (n=57) suffering from gastroenteritis. Among the most common
35 sequence types (STs) we identified were the globally disseminated, host generalist ST-21 clonal
36 complex (CC21) and the poultry specialist CC206, CC464 and CC48. Notably, CC45 and the cattle-
37 specialist CC42 were under-represented with a total absence of CC61. Comparative genomics were
38 used to quantify core and accessory genome sharing among isolates from the same country compared
39 to sharing between countries. Lineage-specific accessory genome sharing was significantly higher
40 among isolates from the same country, particularly CC21 which demonstrated greater local
41 geographical clustering. In contrast, no geographic clustering was noted in either the core or accessory
42 genomes of the CC828, suggesting a highly admixed population. A greater proportion of *C. coli*
43 isolates were multidrug resistant (MDR) compared to *C. jejuni*. This is a significant public health
44 concern as MDR food chain pathogens are difficult to treat and often pose increased mortality risk
45 demanding enhanced prevention strategies in the Egyptian market to combat such a threat.

46

47 **Impact statement**

48 *Campylobacter* is the leading bacterial cause of gastroenteritis worldwide and despite high incidence
49 in low- and middle-income countries, where infection can be fatal, culture-based isolation is rare and
50 the genotypes responsible for disease are seldom identified. Here, we sequenced the genomes of a
51 collection of isolates from clinical cases and potential infection reservoirs from Cairo in Egypt and
52 characterised their genetic diversity. Among the most common genotypes we identified were globally
53 disseminated lineages implicated in human disease worldwide, including the host generalist ST-21
54 clonal complex (CC21) and the poultry specialist genotypes CC206, CC464 and CC48. Notably
55 however, some other globally common genotypes were under-represented or entirely absent from our
56 collection, including those from cattle-specialist lineages, CC42 and CC61. By focussing on specific
57 lineages, we demonstrate that there is increased accessory genome sharing in specific clonal
58 complexes. This increased local sharing of genes may have contributed to a greater proportion of *C.*
59 *coli* isolates possessing antimicrobial resistance determinants that suggest they could be multidrug
60 resistant (MDR). This is a significant public health concern as MDR food chain pathogens are
61 difficult to treat and often pose increased mortality risk demanding enhanced prevention strategies.

62

63 **Data summary**

64 Short read data are available on the NCBI Sequence Read Archive, associated with BioProject
65 PRJNA576513 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576513>). Assembled genomes,
66 supplementary material and additional analysis files are available from FigShare:
67 <https://doi.org/10.6084/m9.figshare.9956597>. Phylogenetic trees can be visualised and manipulated
68 on Microreact for *C. jejuni* (https://next.microreact.org/project/Cjejuni_Egypt) and *C. coli*
69 (https://next.microreact.org/project/Ccoli_Egypt) separately, or combined Cairo and Oxford data with
70 additional PopPunk network clustering (<https://microreact.org/project/Campy-Egypt>).

71

72 Introduction

73 Diarrheal disease is a major cause of child morbidity, growth faltering and mortality in low- and
74 middle-income countries (LMICs) (McCormick and Lang, 2016; Platts-Mills and Kosek, 2014).
75 *Campylobacter* is the most common cause of bacterial gastroenteritis worldwide (Kaakoush et al.,
76 2015) and typically human campylobacteriosis is commonly diagnosed as a disease associated with
77 consumption of contaminated food, especially poultry (Nichols et al., 2012; Sheppard et al., 2009).
78 Extremely high incidence in LMICs, high exposure rates (Lee et al., 2013) and endemism among
79 young children suggests a different epidemiology (Kaakoush et al., 2015; Lanata et al., 2013; J. Liu et
80 al., 2016). Frequent or chronic (re)infection is allied to significant morbidity, cognitive development
81 impairment, and even death (Coker, 2002; Crofts et al., 2018; Kirk et al., 2018; Reed et al., 1996). In
82 Egypt, campylobacteriosis is common and a leading cause of paediatric diarrhoea, with an incidence
83 of 1.2 episodes per year (ElGendy et al., 2018; Rao, 2001) with up to 85% of children infected in their
84 first year (Liu et al., 2012). Despite the high frequency of reported cases of *Campylobacter*-associated
85 diarrhoea in Egypt (ElGendy et al., 2018), there are no detailed surveillance studies on the dominant
86 sequence types and proliferation of genotypes associated with the onset of post-infectious sequelae,
87 such as irritable bowel syndrome (PI-IBS), Guillain-Barré syndrome (GBS) or Miller
88 Fisher syndrome (Wierzba et al., 2008).

89

90 *Campylobacter* species are often part of the gut microbiota of various wild and farmed animals
91 leading to frequent contamination of human food products (Asuming-Bediako et al., 2019; Waite and
92 Taylor, 2015). In Egypt, farming practices can lack adequate biosecurity and regulation. Only limited
93 studies have reported the prevalence and distribution of *Campylobacter* in Egyptian
94 campylobacteriosis cases (Kaakoush et al., 2015) and little is known of the dominant source reservoirs
95 driving infection and transmission. In Europe, potential source reservoirs have been identified through
96 source attribution studies, with poultry products regarded as the primary source of infection (Facciola
97 et al., 2017; Mossong et al., 2016; Sheppard et al., 2009; Thépault et al., 2018). Host-adaptation of
98 *Campylobacter* to a wide-range of hosts is reflected in its population structure (Colles and Maiden,

99 2012; Dearlove et al., 2016; Griekspoor et al., 2013; Méric et al., 2018; Sheppard et al., 2014), with
100 many lineages common in human infection able to infect multiple host species. These host generalist
101 lineages include *C. jejuni* ST-21, ST-45 clonal complexes and the *C. coli* ST-828 complex (Dearlove
102 et al., 2016; Mossong et al., 2016). Other genotypes are only found in a single reservoir species, often
103 associated with global poultry or cattle production. Host specialist clonal complexes common in
104 human disease includes the poultry-associated ST-353, ST354 and ST257 (Berthenet et al., 2019;
105 Sheppard et al., 2009) and cattle specialist ST-61 (French et al., 2005; Mourkas et al., 2019).

106

107 Human infection in developed countries is usually sporadic and self-limiting, not requiring treatment
108 with antibiotics. However global rates of antimicrobial resistance are rising (Mourkas et al., 2019;
109 Zhao et al., 2016) in line with other Gram negative gastrointestinal pathogens (Tam et al., 2012; CDC,
110 2020). Widespread agricultural usage has driven the proliferation of tetracycline resistance through its
111 use as a growth promoter (Abdi Hachesoo et al., 2014; Inglis et al., 2019). In particular, *C. coli* has
112 shown an ability to acquire erythromycin resistance genes from other species (Mourkas et al., 2019).
113 This has not been explored for Egyptian *Campylobacter* isolates, where agricultural antibiotic usage is
114 poorly regulated (Dahshan et al., 2015) and self-medication for gastrointestinal disease is common
115 (Abd El-Tawab et al., 2018; Sabry et al., 2014). Global differences in the use of quinolones is likely
116 responsible for the geographical differences observed in quinolone resistance (Luangtongkum et al.,
117 2009; Pascoe et al., 2017; Zollner-Schwetz and Krause, 2015).

118

119 We have sequenced 112 *Campylobacter* isolates collected from patients and food of animal source
120 (i.e., broiler chicken carcasses and dairy products) in Cairo over a year to determine the most
121 prevalent *Campylobacter* genotypes causing disease in Egypt. By screening the genome content,
122 including known AMR determinants we provide a better understanding of the local population
123 structure to guide disease intervention in Egypt. This study provides a basis for considering complex
124 transmission networks in LMICs and highlights the role of globally transmitted *Campylobacter*
125 lineages and the emergence of (horizontally acquired) antimicrobial resistance.

126 **Methods**

127 ***Ethical approval***

128 The study represents a retrospective study that involved sequencing the genomes of a historical strain
129 collection and no patient data collection was involved in this study. Ethical approval was granted from
130 the respective ethics committee in the Egyptian central directorate of research and health development
131 before conducting the study.

132

133 ***Isolate collection***

134 In total, 112 *Campylobacter* isolates were collected in Cairo, Egypt from September 2017 to
135 December 2018, including 31 isolates from broiler carcasses, 24 isolates from milk and dairy
136 products, and 57 clinical isolates. Clinical isolates were recovered from stool samples of patients
137 admitted to hospitals in downtown Cairo suffering from gastroenteritis symptoms. A questionnaire
138 was distributed to all admitted patients requesting details on clinical presentation (e.g., duration of
139 illness, symptoms, medication prescribed), dietary record of the previous 2 weeks, including
140 consumption of specific or undercooked meats, unpasteurized milk, exposure to animal manure or
141 faeces, and any retail outlets commonly used by patients for food consumption prior to the onset of
142 illness. A random sampling approach was then used to include food samples from stores in the study
143 region that were commonly listed in the questionnaire.

144

145 ***Sample culturing and whole genome sequencing***

146 The isolation and enumeration of *Campylobacter* strains from different food matrices was performed
147 according to the ISO 10272-1 (Enrichment Method; Detection of *Campylobacter* spp. after Selective
148 Enrichment). All isolates were sub-cultured from -80°C frozen stocks onto Mueller-Hinton agar
149 (Oxoid, United Kingdom). Plates were incubated at $42 \pm 1^{\circ}\text{C}$ under anaerobic conditions using
150 AnaeroGen™ 2.5L Sachets (Oxoid, United Kingdom). Genomic DNA was extracted from 112
151 Egyptian isolates using the QIAamp DNA Mini Kit (QIAGEN, Crawley, UK), according to
152 manufacturer's instructions and DNA concentrations were quantified using a Nanodrop

153 spectrophotometer before genome sequencing using an Illumina MiSeq (California, USA). Nextera
154 XT libraries (Illumina, California, USA) were prepared following manufacturer's protocols and short
155 paired-end reads were sequenced using 2×300 bp paired end v3 reagent kit (Illumina).

156

157 ***Genome datasets***

158 Genomes were assembled *de novo* using SPAdes (version 3.8.0; Bankevich et al. 2012). The average
159 number of contigs was 72 (range: 12–471) for an average total assembled sequence size of 1.70 Mbp
160 (range: 1.56–1.86). The average N50 contig length (L50) was 14,577 (range: 3,794–55,912) and the
161 average GC content was 30.8 % (range: 30.5–31.6). Short read data are available on the NCBI short
162 read archive (SRA), associated with BioProject PRJNA576513. Assembled genomes and
163 supplementary material are available from FigShare (doi:10.6084/m9.figshare.9956597; individual
164 accession numbers and assembled genome statistics in **Supplementary Table S1**). We augmented
165 our collection by assembling a context dataset of previously published isolates (n=204) to represent
166 the known diversity of *C. jejuni* and *C. coli* (Calland et al., 2020; Sheppard et al., 2010, 2013, 2014).
167 In addition, we also compared our single city survey with a previously published survey from Oxford
168 in the UK (n=874 isolates collected over 1 year; Cody et al. 2012). Isolate genomes were archived in
169 BIGSdb and MLST sequence types (STs) derived through BLAST comparison with the pubMLST
170 database (Dingle et al., 2001; Jolley et al., 2018; Jolley and Maiden, 2010; Sheppard et al., 2012).
171 Simpson's index of ST diversity was calculated for the Cairo and Oxford datasets using the equation:

$$D = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

172 Where *n* is the number of isolates of each sequence type and *N* is the total number of isolates
173 (Grundmann et al., 2001).

174

175 ***Core and accessory genome characterisation***

176 Alignments were made from concatenated gene sequences of all core genes (found in ≥95% isolates)
177 using MAFFT (version 7; Katoh and Standley 2013) on a gene-by-gene basis. Separate maximum-
178 likelihood phylogenies were constructed with a GTR+I+G substitution model and ultra-fast

179 bootstrapping (1000 bootstraps) (Hoang et al., 2018) implemented in IQ-TREE (version 1.6.8;
180 Nguyen et al. 2015) for *C. jejuni* (n=1,048) and *C. coli* (n=132) and visualized on Microreact
181 (https://next.microreact.org/project/Cjejuni_Egypt; https://next.microreact.org/project/Ccoli_Egypt)
182 (Argimón et al., 2016).

183

184 All unique genes present in at least one isolate (the pangenome) were identified by automated
185 annotation using PROKKA (version 1.13; Seemann 2014) followed by PIRATE, a pangenomics tool
186 that allows for orthologue gene clustering in bacteria (Bayliss et al., 2019). We defined genes in
187 PIRATE using a wide range of amino acid percentage sequence identity thresholds for Markov
188 Cluster algorithm (MCL) clustering (45, 50, 60, 70, 80, 90, 95, 98). Genes in the pangenome were
189 ordered initially using the NCTC 11168 reference followed by the order defined in PIRATE based on
190 gene synteny and frequency (Gundogdu et al., 2007; Pascoe et al., 2019). As described previously, a
191 matrix was produced summarizing the presence/absence and allelic diversity of every gene in the
192 pangenome list, with core genes defined as present in 95% of the genomes and accessory genes as
193 present in at least one isolate (**Supplementary table S2**) (Méric et al., 2014). Pairwise core and
194 accessory genome distances were compared using PopPunk (version 2.2.0; Lees et al. 2019) which
195 uses pairwise nucleotide k-mer comparisons to distinguish shared sequence and gene content to
196 identify divergence of the accessory genome in relation to the core genome. A two-component
197 Gaussian mixture model was used to construct a network to define clusters, comparable to other
198 *Campylobacter* studies (Components: 41; density 0.0579; transitivity: 0.9518; score: 0.8907) (Pascoe
199 et al. 2020).

200

201 Core genome variation between isolates was quantified by calculating the pairwise average nucleotide
202 identity (ANI) of all (n=112+874) *Campylobacter* genomes using FastANI v.1.058 (Jain et al., 2018).
203 The gene presence matrix produced by PIRATE was used to generate a heatmap of shared pairwise
204 accessory genome genes. Averages were calculated for within and between country comparisons in
205 addition to focussed analysis on the ST21 (*C. jejuni*) and ST828 (*C. coli*) clonal complexes.
206 Antimicrobial resistance genes and putative virulence genes were detected through comparison with

207 reference nucleotide sequences using ABRicate (version 0.8) (<https://github.com/tseemann/abricate>)
208 and the NCBI database (Chen et al., 2005; NCBI Resource Coordinators, 2013). Point mutations
209 related to antibiotic resistance genes were identified by PointFinder (Zankari et al., 2017) using the
210 STAR-AMR software package (<https://github.com/phac-nml/staramr>) (**Supplementary table S3**).
211

212 Results

213 *Globally circulating genotypes among Egyptian Campylobacter isolates*

214 We sequenced and characterized a collection of *Campylobacter spp.* isolates (n=112) from clinical
215 cases, broiler carcasses and dairy products collected over a 14-month sampling period in Cairo, Egypt
216 (**Figure 1A; Supplementary table S1**). Isolate genotypes were compared with all genomes deposited
217 in the pubMLST database (97,012 profiles, data accessed 17th February 2020) and ranked according to
218 how frequently they were found associated with human disease (**Figure 1B**). Egyptian *C. jejuni*
219 isolates belonged to 15 clonal complexes (CCs) with a diverse assemblage of STs. Nearly half of the
220 isolates (n = 29, 47%) were from common lineages, isolated many times before and recorded in
221 pubMLST (>50 MLST profiles; **Figure 1B**), including the globally disseminated lineages of ST-
222 21CC (n=37; 41%), ST-206 CC (n=10; 11%) and ST-464CC (n=7; 8%) the most abundant. Several
223 other poultry-associated clonal complexes, which are common in human disease (Berthenet et al.,
224 2019; Sheppard et al., 2009), including ST-353 (n=3, 3.2%), ST-354 (n=4, 4.3%) and ST-257
225 (n=4, 4.3%) were identified. Other globally disseminated lineages were found less often in Egypt
226 (n<=2) i.e., ST460 (n=2), ST1034 (n=2), ST42 (n=1), ST-45 CC (n=1), ST573
227 (n=1), ST574 (n=1) and ST658 (n=1) (Colles et al., 2010; Olkkola et al., 2016).

228

229 ***Local sequence types***

230 Comparison with a collection representing the known genetic diversity of *C. jejuni* and *C. coli*
231 identified some common STs (>1,000 profiles in pubMLST) that were completely absent in our
232 Egyptian collection, i.e., ST-53, ST-829 (*C. coli*), ST-22, ST-61, ST-51, ST-1068 (*C. jejuni*) (**Figure**
233 **1CD**). Two isolates belonging to ST-1287CC, a genotype that has previously been isolated from
234 poultry and the environment (Magnússon et al., 2011), was observed exclusively among our Egyptian
235 isolates, yet absent in UK and genetic context datasets. Furthermore, there were also some STs
236 belonging to ST-21CC that were found in Egyptian isolate collection (n=>3) that are rare in global
237 collections (<100 profiles in pubMLST), i.e., ST-1519 (n=4), ST-3769 (n=3). It was also observed
238 that more *C. coli* was found among Egyptian clinical isolates than is typically observed, specifically
239 the *C. coli* lineage ST-828 CC 90.4% *C. coli* isolates (19/21) belonged to the ST-828 CC within the
240 Egyptian dataset and two *C. coli* isolates with unassigned CC of sequence types, ST-7951 and ST-
241 1681. Three rare STs belonging to ST-828 CC were exclusively found in Egypt dataset which are ST-
242 1058 (n=1), ST-1059 (n=1), and ST-7950 (n=1).

243

244 ***Increased sharing of accessory genes contributes to a local gene pool***

245 Our Egyptian dataset was compared directly with a previously published study of a single city, ~1-
246 year survey from Oxford in the UK (Cody et al., 2012). Both populations were similarly diverse,
247 specifically there were 50 STs (16 CCs) among the Egyptian isolate collection, with a Simpson's
248 diversity index of 0.817, compared to 205 STs (32 CCs) among the Oxford collection of genomes
249 (Simpson's diversity index = 0.895; **Figure 1CD**). We used PIRATE to construct a pan-genome of all
250 Egyptian and Oxford isolates (n=986). Consistent with other studies, we identified an open
251 pangenome, meaning that the number of genes in the pangenome continues to increase with each
252 additionally sequenced isolate. Accessory genes represented nearly three-quarters of the pangenome
253 (3,410 genes; 74% of pangenome) with a quarter of the genes identified (1,225, 26%) considered core
254 genes present in 95% or more of the isolates. Pairwise comparison of the core nucleotide sequence
255 (%ANI) and accessory genome sharing of all isolates reflected the clonal frame, with clusters of
256 closely related isolates sharing a large percentage of ANI (**Figure 2AB**). Direct comparison between

257 the Oxford and Cairo datasets suggested an increase in within-country, local accessory gene sharing
258 (**Figure 2CD**). The structured clustering of pairwise comparisons of shared accessory genes
259 suggested that this may vary between lineages and visualization of the differences in the distribution
260 of pairwise genomic distances with PopPUNK also pointed towards lineage-specific shared gene
261 pools (**Figure 2E**). Host generalist clonal complex isolates clustered closer together than the more
262 isolated host-specific isolates. This included the two most common clonal complexes identified in our
263 Cairo collection, ST-21CC and ST-828CC, which were investigated further (**Figure 2F**).

264

265 *Locally diverged sequence types within the globally disseminated ST-21 clonal complexes*

266 As one might expect of within lineage (clonal complex) comparisons, all ST-21CC isolates shared
267 more than 99% core genome nucleotide identity and shared more accessory genes than the population
268 average (852 genes; **Figure 2CF**) and significantly more genes were shared between isolates from the
269 same country (*t*-test with Welch correction; $p < 0.0001$). A maximum-likelihood phylogeny of all
270 CC21 isolates ($n=251$), the most common clonal complex identified in our collection from Cairo,
271 identified geography-specific clusters of isolates (**Figure 3A**). These clones also clustered together
272 when visualizing the distribution of pairwise genomic distances with PopPUNK (**Figure 3B**). While
273 some specific STs were common in both Oxford and Cairo (ST21 and ST50), others were much more
274 common in one specific location, e.g., ST-53 in Oxford, and ST-1519 and ST-3769 in Cairo (**Figure**
275 **3C**). There was also evidence that some lineages had enhanced AMR (**Figure 3D**). While the ST-50
276 genotype is very common and has been reported more than 3,900 times in pubMLST from 40
277 countries, this among the first reports from Africa. In both Oxford and Cairo datasets, ST-50 was
278 often predicted to be MDR. ST-21 is also very common, with more than 4,000 reports from 33
279 countries in pubMLST but was much less likely to be MDR. Four isolates of the Cairo specific ST-
280 3769 also represented a high proportion of MDR (**Figure 3E**).

281

282 *Extensive multi-drug resistance in local C. coli sequence types*

283 Greater admixture was noted between UK and Egyptian ST-828CC isolates than for ST-21CC – no
284 geographic clustering was observed in either the core or accessory genomes (**Figure 4AB**). However,

285 only ST-827 was common in both datasets (**Figure 4C**). Several STs were found in the Oxford
286 dataset that were not identified in Cairo, including the frequently isolated STs -829, -828, -855, 962, -
287 1145 and -5734. Several lineages were highly resistant to lincosamides, with more than half the
288 isolates from ST-828, ST-830 and ST-872 predicted to be resistant (**Figure 4D**). All isolates from
289 ST828 and ST-872 were also predicted to be resistant to chloramphenicol. Overall, *C. coli* isolates (6
290 of 105, 5.7%) were far more likely to be considered MDR than *C. jejuni* isolates (6 of 876, 0.68%)
291 and ST-828 complex isolates from Cairo (2 of 19, 10.5%) demonstrated much higher rates of MDR
292 than in Oxford (3 of 77, 3.8%; **Figure 4E**).

293

294 *Antimicrobial resistance genes are distributed across isolates*

295 In characterization of the resistome, each isolate genome was screened for the presence of genes
296 associated with AMR. In Egypt, for *C. jejuni*, the average number of AMR genes per isolate was 6.66,
297 comparable to 6.52 for *C. coli*. In Egypt, the presence of the *tet(O)* gene, conferring tetracycline
298 resistance, was higher in *C. coli* than *C. jejuni* (76% and 43% respectively). This pattern contrasts
299 with Oxford where 41.7% of *C. jejuni* but only 35.3% of *C. coli* isolates were found to harbor *tet(O)*.
300 Whilst a low proportion of Egyptian isolates (6.7%) contained the *blaOXA-61* gene, associated with
301 β -lactam resistance, alternative alleles including *blaOXA-450* and *blaOXA-605* were abundant. In
302 respect to lineage association with genes, in Egypt the ST-21 clonal complex had a high prevalence of
303 genes associated with β -lactam resistance (particularly the *blaOXA-193*, *blaOXA-450* and *blaOXA-*
304 *605* alleles). The *blaOXA-465* allele was closely related to ST-1034. Furthermore, *blaOXA-61* was
305 closely associated with ST-48 (**Figure 3D**). All of these patterns were reflected amongst the Oxford
306 isolates. However, numerous genes (including *aadE*, *Ant6-la* and *blaOXA-451*) were found amongst
307 distant lineages. The multi-drug efflux pump encoded by a three-gene operon (*cmeABC*) was
308 abundant amongst isolates (n=87,74%) – although an absence of the repressor gene *cmeR* in *C. coli*
309 was observed.

310

311 Whilst the average number of resistance genes per isolate was comparable for *C. jejuni* in Egypt, this
312 analysis indicated that *C. coli* held a greater breadth of genes across classes of antimicrobials. Hence,

313 the proportion of MDR isolates, considered when an isolate is resistant to at least three classes, was
314 28% for *C. coli* compared to 1% for *C. jejuni* (EFSA, 2021). The majority (88%) of MDR isolates in
315 Egypt were *C. coli*, despite *C. coli* representing about a fifth of the dataset. In other words, a greater
316 proportion of *C. coli* isolates were MDR. In Oxford, half of MDR isolates were *C. coli*, whilst in this
317 case representing less than one tenth of the dataset. The *C. jejuni* isolates that were MDR, were all
318 host generalists – ST-21, ST-48 or ST-206. Amongst Egyptian isolates, genes including *aad9*, *aadE*,
319 *aadE-Cc*, *ant(6)-Ia*, *aph(2'')-If*, *aph(3')-III* and *aph(3')-IIIa* associated with aminoglycoside resistance,
320 were almost exclusively associated with *C. coli*, particularly MDR *C. coli*. This association was not as
321 strong in Oxford. Regarding specific genes and host associations, aminoglycoside resistance-
322 associated genes were infrequent amongst isolates from chicken or dairy products. *ant(6)-Ia* for
323 example, was solely found in human samples. In turn, few isolates from chicken and dairy products
324 were MDR (only 12.5% of MDR isolates was from chicken).
325

326 Discussion

327 Diarrheal disease is a major threat to human health and the second leading cause of death in children
328 under five years' old LMICs (Lanata et al., 2013). Campylobacteriosis is a major cause of diarrheal
329 disease worldwide (Amour et al., 2016; ElGendy et al., 2018; Lee et al., 2013) but, despite the
330 potential importance, little is known about *Campylobacter* in countries where it potentially poses the
331 greatest health risk. As studies begin to take a worldview of *Campylobacter* epidemiology and
332 transmission (Mottet and Tempio, 2017), we describe globally disseminated agriculture-associated
333 disease-causing lineages based on core and accessory genome content, with evidence that local
334 accessory genome sharing driving acquisition of AMR genes in specific lineages.

335

336 The Egyptian *Campylobacter* isolates included a diverse set of STs, including common disease-
337 causing lineages and regional STs, that have rarely been reported from other parts of the world.
338 Industrialized agriculture globalization has dispersed livestock worldwide (Mottet and Tempio, 2017),
339 expanding the geographical range of *C. jejuni*. This is evident in the Egyptian collection as two of the
340 most predominant genotypes belonged to the ST-21 and ST-206 clonal complexes (Figure 1D). These
341 two host generalist clonal complexes have been extensively reported worldwide and frequently
342 isolated from various reservoir hosts, including human clinical samples (Berthenet et al., 2019; Dingle
343 et al., 2001; Grove-White et al., 2011; Mossong et al., 2016; Sheppard et al., 2009; Suerbaum et al.,
344 2001). The ST21-CC exhibits considerable genome plasticity with a clear association with several
345 virulence genes and resistance to various antimicrobial agents (Aksomaitiene et al., 2019; Gripp et al.,
346 2011; Habib et al., 2010; Wieczorek et al., 2017; T. Zhang et al., 2016). Poultry-associated clonal
347 complexes, ST-206, ST-464, ST-48, ST-257 and ST-354 were also common among the Egyptian
348 isolates, all of which are among the most prevalent clonal complexes isolated in Europe (Colles et al.,
349 2011; Elhadidy et al., 2018; Fiedoruk et al., 2019).

350

351 Further comparison of isolate genotypes collected in Cairo with a large global collection revealed the
352 absence of certain lineages, most notably the lack of the cattle-associated genotype, ST-61 (Dingle et

353 al., 2002; Mourkas et al., 2020). There was only one isolate, of dairy product origin, that could be
354 attributed to a cattle-specialist clonal complex (ST-42), which is unexpected as several (n=24) isolates
355 were sampled from dairy products. *Campylobacter* isolates from cattle have predominantly been
356 sampled from meat, milk products and fecal sources (n=2,726 in pubMLST; Kwan et al., 2008;
357 Mourkas et al., 2020; Epping et al., 2021). Suggesting that dairy products isolates might represent a
358 different source population in Egypt.

359

360 There were also no isolates belonging to the ST-22 CC, a particularly high risk lineage which is
361 commonly found among patients with post-infectious complications of campylobacteriosis, such as
362 GBS and IBS (Revez et al., 2011; Peters et al., 2021). Although one isolate in our collection was from
363 ST-45 CC, this host generalist clonal complex is often one of the most commonly isolates lineages in
364 clinical surveillance studies worldwide (De Haan et al., 2010; Sheppard et al., 2009; Shin et al., 2013;
365 Sopwith et al., 2008). Notably however, it is often absent (or under-represented) in studies conducted
366 in LMICs (Pascoe et al., 2020; Sarhangi et al., 2021). This is consistent with observations from other
367 LMICs, where local differences in disease epidemiology are reflected by the absence of common
368 *Campylobacter* lineages, and the presence of rare or unique sequence types (Graham et al., 2016;
369 Pascoe et al., 2020; Prachantasena et al., 2016; P. Zhang et al., 2020). Among our Egyptian isolates
370 the ST-1287 clonal complex (n=2) has been reported less than 4 times from other parts of the world
371 (Colles et al., 2011; de Haan et al., 2010; Ramonaite et al., 2014; P. Zhang et al., 2020).

372

373 Geographical differences have been noted in ST-21CC (Kärenlampi et al., 2007; Kovanen et al.,
374 2014; Olkkola et al., 2016; Pascoe et al., 2017; Wallace et al., 2021). ST-21 CC isolates are among
375 the most common *C. jejuni* genotypes isolated worldwide, with one quarter of *C. jejuni* isolates
376 recorded in the pubMLST database are ST21 CC. Isolates of the ST-50 sequence type (n= 3,915)
377 alone have been sampled from 6 continents and 44 countries, although this will be their first report
378 from Africa (Jolley et al., 2018). Our Egyptian ST-50 isolates do cluster together on a ML phylogeny
379 of ST-21 CC isolates and away from the Oxford ST-21 CC when grouped by PopPunk. Two sequence
380 types were unique to Egypt, ST-1519 and ST-3769, with nearly 10% of the ST-3769 isolates were

381 MDR. A slightly greater proportion of the Egyptian ST-50 isolates were also MDR, although this
382 sequence type has been observed to be MDR in other parts of the world (Elhadidy et al., 2020).

383

384 The *C. coli* ST-828 clonal complex did not show as much geographical segregation, and when
385 grouping our Egyptian isolates by core and accessory genome distances they clustered with the UK
386 isolates, despite several STs being isolated in only one of the datasets. STs found in the Egyptian
387 dataset were more often MDR than UK isolates, and overall *C. coli* from Cairo were far more MDR
388 than *C. coli* isolates from developed countries (Du et al., 2018; Gharbi et al., 2018; Mourkas et al.,
389 2019). The most compelling clarification for such abundance could be that *C. coli* of ST-828 CC have
390 a great recombination potential besides the accumulation of *C. jejuni* DNA throughout the genome of
391 this lineage which could have led to the acquisition of multiple AMR genes (Sheppard et al., 2008,
392 2013).

393

394 Overall, there is a clear evidence of local sharing and recent acquisition of accessory gene content of
395 AMR genes within the Egyptian isolates. Specifically, pairwise clustering of isolates by core and
396 accessory genome distances recapitulated clusters according to ST and clonal complex (**Figure 2**),
397 however most Egyptian isolates were more tightly clustered than the Oxford dataset, consistent with
398 shared acquisition of accessory genes. Overall, ANI and shared accessory genes were similar between
399 Oxford and Egyptian isolates (per isolate), however the two most common clonal complexes found in
400 our Cairo dataset demonstrated greater sharing of accessory genes, indicative of a shared gene pool.
401 Our study suggested that while geographical partitioning doesn't impact the composition of the core
402 genome, represented by the shared STs and CCs, the accessory genome is influenced. Within the
403 Egyptian isolates, the most prevalent *C. jejuni* genotypes (ST-21CC and ST-206CC) showed clear
404 evidence of transmission of MDR determinants among lineages. Multiple factors could influence this,
405 such as livestock and food production practices and the segregation of MDR isoaltes. However,
406 selective pressure for MDR is clearly attributable to antibiotic usage and potentially zoonotic
407 transmissions as well as the rate of horizontal gene transfer (Fiedoruk et al., 2019). Our study
408 provides evidence to support programs aimed at improved antibiotic stewardship in clinical and

409 veterinary settings. With strict control measures, and an understanding of transmission of strains from
410 animal reservoirs through the food production chain, it may be possible to reduce contamination with
411 MDR *Campylobacter* in Egypt.
412

413 **Author statements**

414 **Author contributions**

415 SM, JKC, BP, ME and SKS designed the study and wrote the paper.

416 JKC, BP, EM, GF, CL, HW performed genomic analysis.

417 BP, CL and MDH sequenced and assembled genomes.

418 All authors contributed and approved the final manuscript.

419

420 **Conflict of interest**

421 All authors declare no conflict of interest.

422

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431

432 **Ethical approval**

433 The study represents a retrospective study that involved sequencing the genomes of a historical strain

434 collection and no patient data collection was involved in this study. Ethical approval was granted from

435 the respective ethics committee in the Egyptian central directorate of research and health development

436 before conducting the study.

437

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439

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854 Tables and Figures

855 **Figure 1:** (A) Demographic data for Cairo, Egypt from which we collected *Campylobacter spp.*
856 isolates (n=112; red circles) from clinical cases, broiler carcasses and dairy products collected over a
857 14-month sampling period. Our collection was compared to a similar published survey from Oxford,
858 UK (n=874; green circles; Cody et al. 2012) and isolates from pubMLST.org (n=204; grey circles) for
859 additional genetic context. (B) Clonal complexes (CCs) of isolates collected from Cairo were ranked
860 according to the frequency in our local dataset and how often they have been sampled from human
861 disease isolates (data from pubMLST; <https://pubmlst.org/>). Alignments were made from
862 concatenated gene sequences of all core genes (found in $\geq 95\%$ isolates) using MAFFT (version 7;
863 Katoh and Standley 2013) on a gene-by-gene basis. Separate maximum-likelihood phylogenies were
864 constructed with a GTR+I+G substitution model and ultra-fast bootstrapping (1000 bootstraps)
865 (Hoang et al., 2018) implemented in IQ-TREE (version 1.6.8; Nguyen et al. 2015) for (C) *C. jejuni*
866 (n=1,048) and (D) *C. coli* (n=132) and visualized on Microreact
867 (https://next.microreact.org/project/Cjejuni_Egypt; https://next.microreact.org/project/Ccoli_Egypt)
868 (Argimón et al., 2016).

869

870 **Figure 2:** (A) Core genome variation between isolates was quantified by calculating the pairwise
871 average nucleotide identity (ANI) of all UK and Oxford *Campylobacter* genomes (n=112+874) using
872 FastANI v.1.058 (Jain et al., 2018). (B) The ANI for each isolate was estimated and averages
873 compared within and between countries. (C) The gene presence matrix produced by PIRATE was
874 used to generate a heatmap of shared pairwise accessory genome genes. (D) Averages were calculated
875 for within and between country. (E) Clustering of pairwise core and accessory genome distances were
876 compared using PopPunk. Interactive visualisation on Microreact:
877 <https://microreact.org/project/Campy-Egypt>. (F) Comparisons of within and between country ANI
878 and accessory gene sharing were also analysed for our two most common Egyptian lineages, ST21 (*C.*
879 *jejuni*) and ST828 (*C. coli*) clonal complexes.

880

881 **Figure 3:** (A) Sub-tree of all Egyptian and UK ST21 clonal complex (CC21) isolates (n=251).
882 Common sequence types are annotated and ST50 (yellow) and ST21 (green) are highlighted. (B)
883 Within clonal complex clustering of pairwise core and accessory genome distances with PopPunk. (C)
884 Prevalence of the most common sequence types found within CC21. (D) Prevalence of AMR
885 determinants grouped by antibiotic class for each CC21 ST. (E) Prevalence of MDR isolates (AMR
886 determinants for three or more antibiotic classes) in CC21 STs.

887

888 **Figure 4:** (A) Sub-tree of all Egyptian and UK ST828 clonal complex (CC828) isolates (n=94).
889 Common sequence types are annotated and ST827 (orange) is highlighted. (B) Within clonal complex
890 clustering of pairwise core and accessory genome distances with PopPunk. (C) Prevalence of the most
891 common sequence types found within CC828. (D) Prevalence of AMR determinants grouped by
892 antibiotic class for each CC828 ST. (E) Prevalence of MDR isolates (AMR determinants for three or
893 more antibiotic classes) in CC828 STs.

894

895 **Supplementary information**

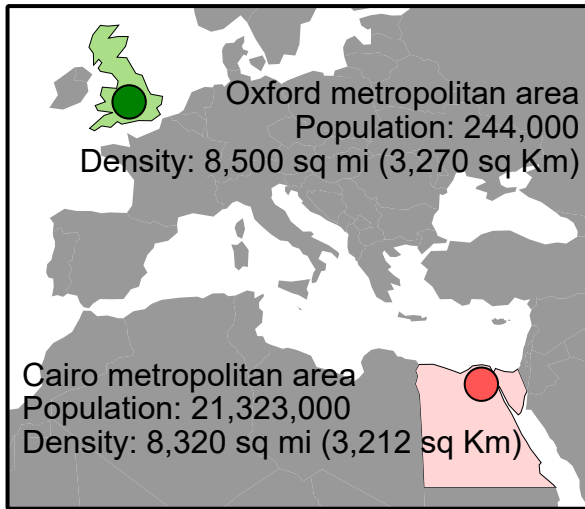
896 **Supplementary table 1:** Summary of isolate collection data and genome statistics

897 **Supplementary table 2:** Summary PIRATE core and accessory genome statistics.

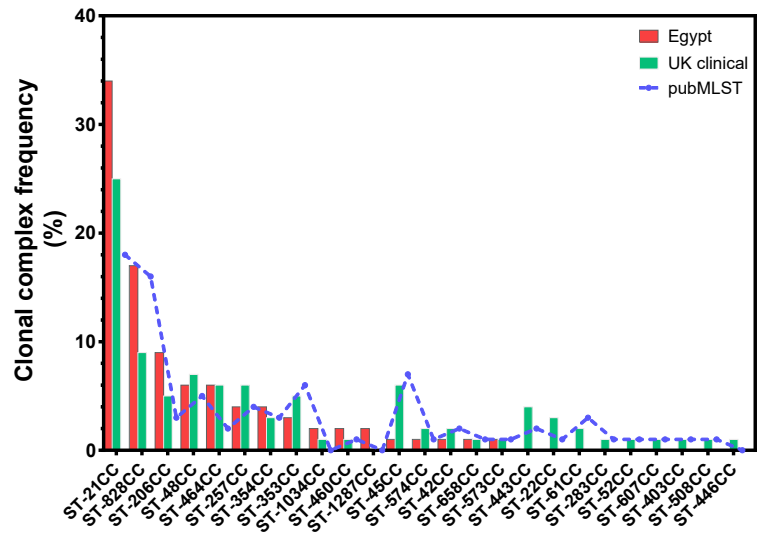
898 **Supplementary table 3:** Summary of AMR genes identified by comparison with the NCBI database
899 and pointfinder.

900

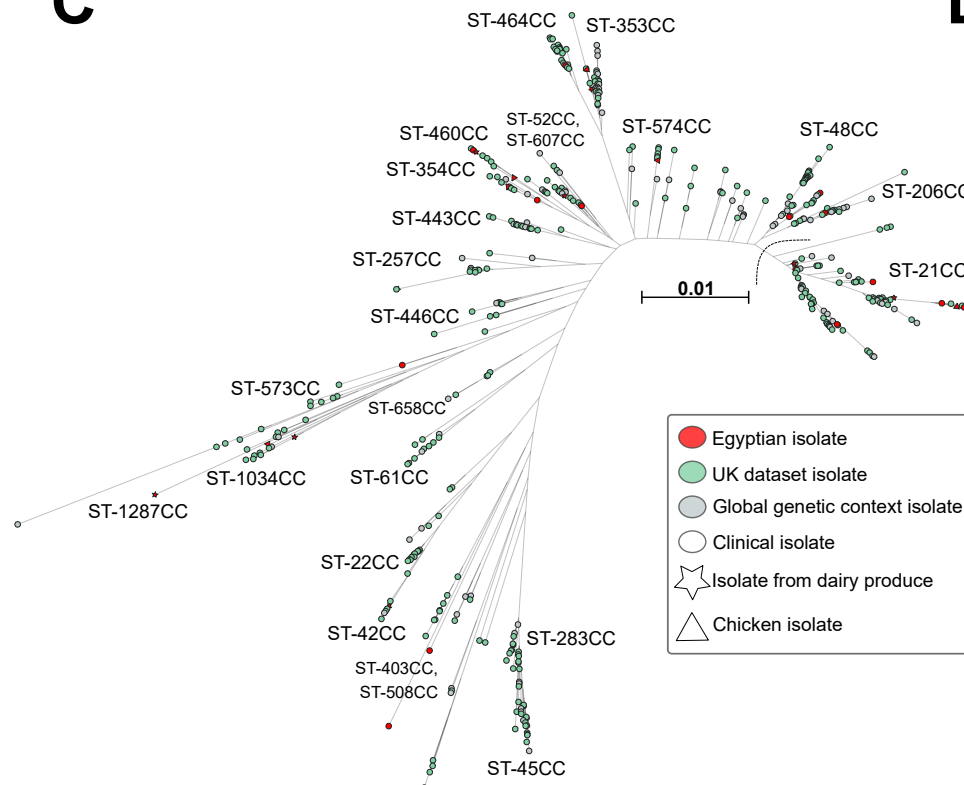
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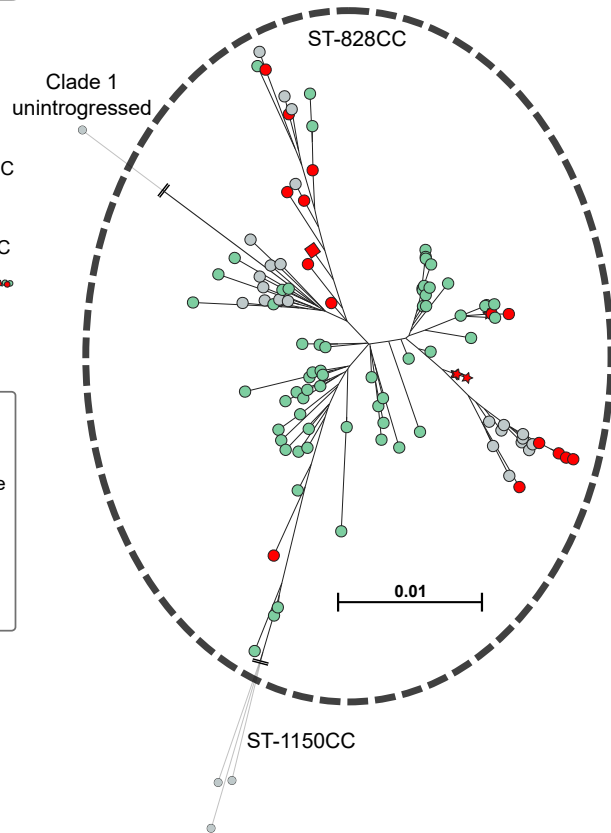
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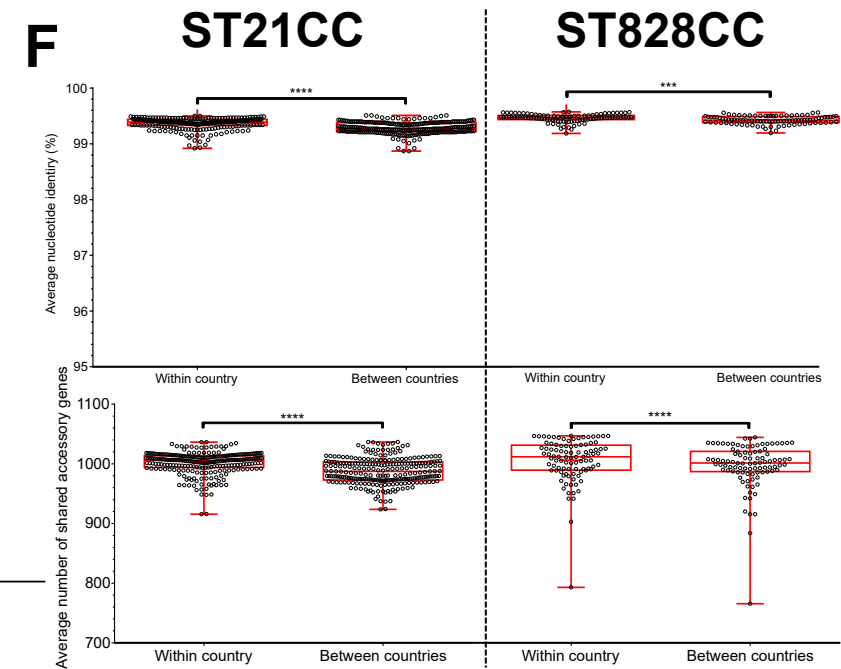
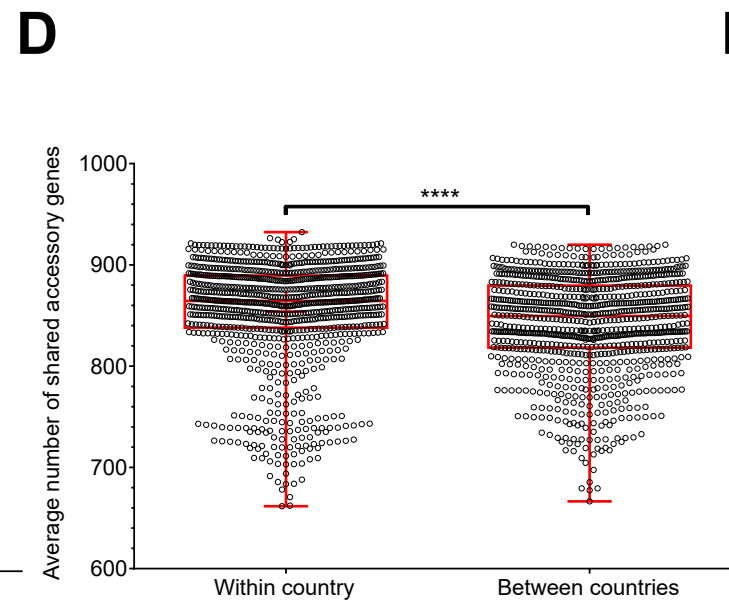
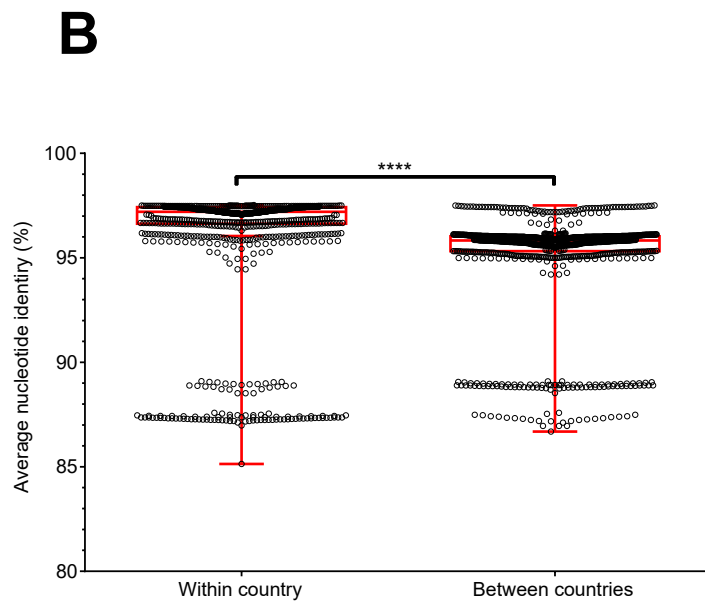
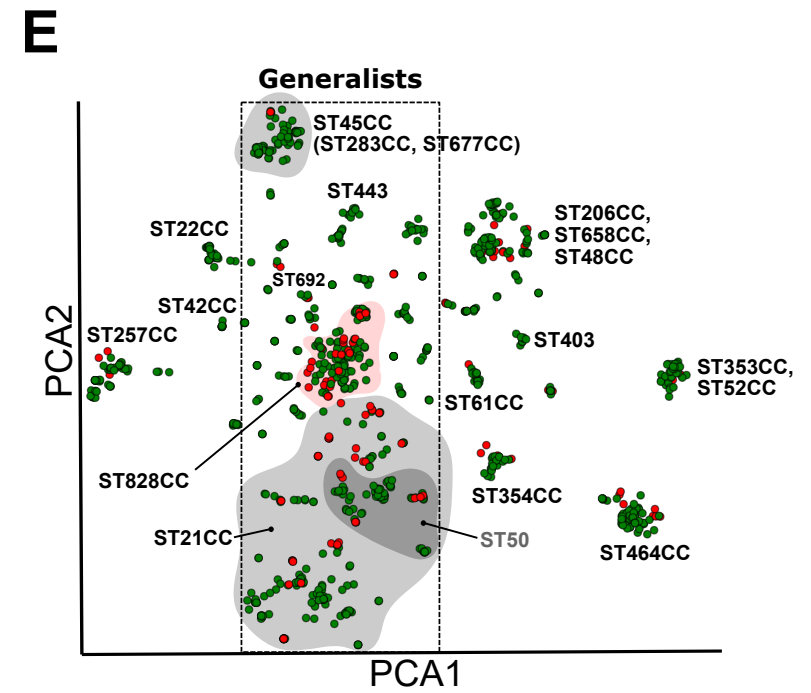
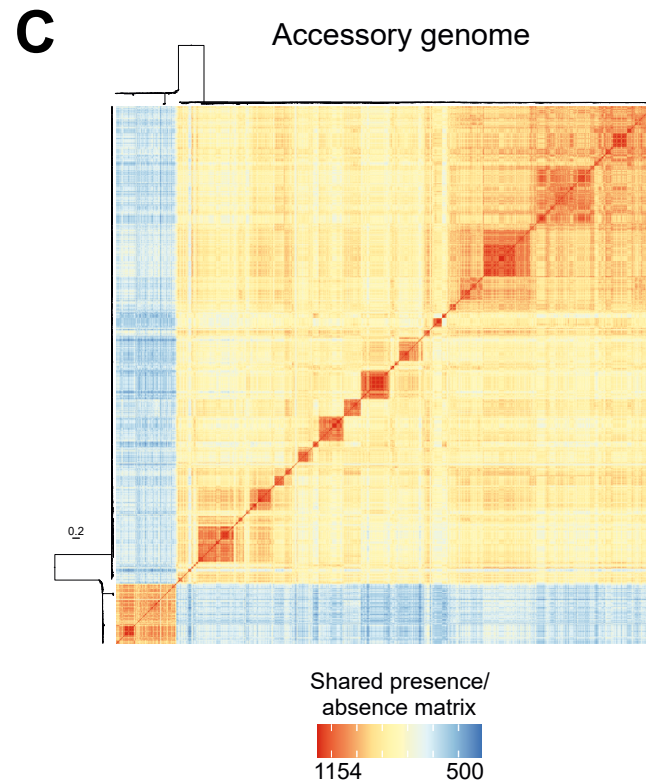
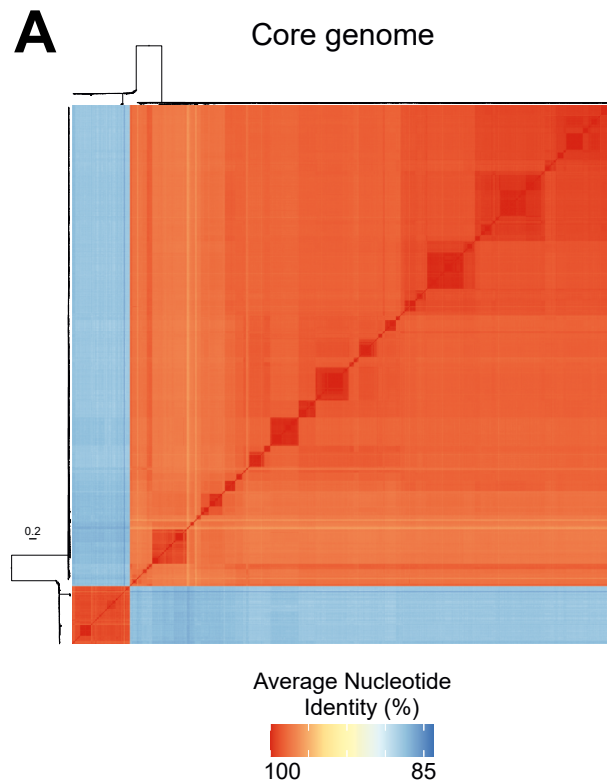
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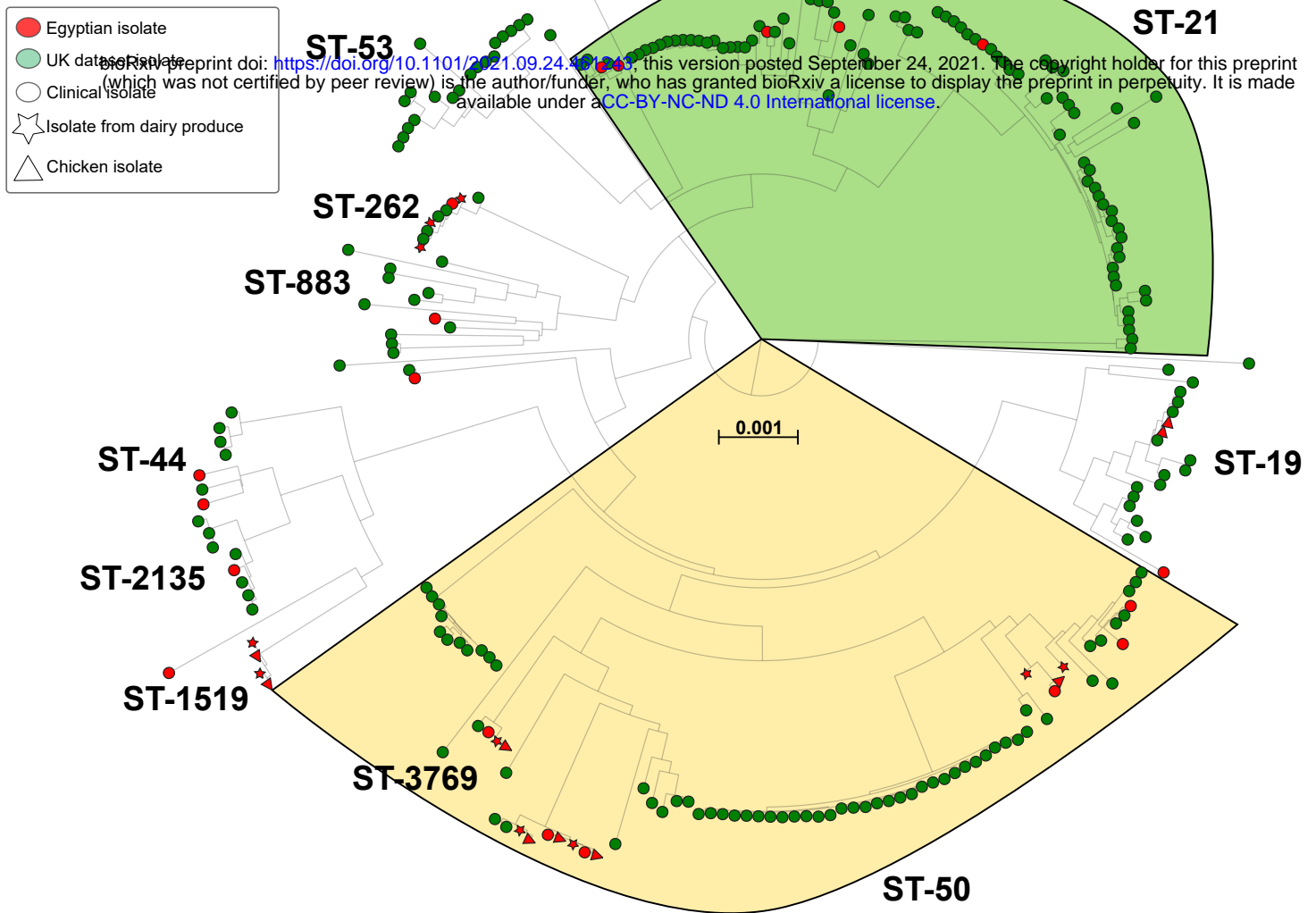
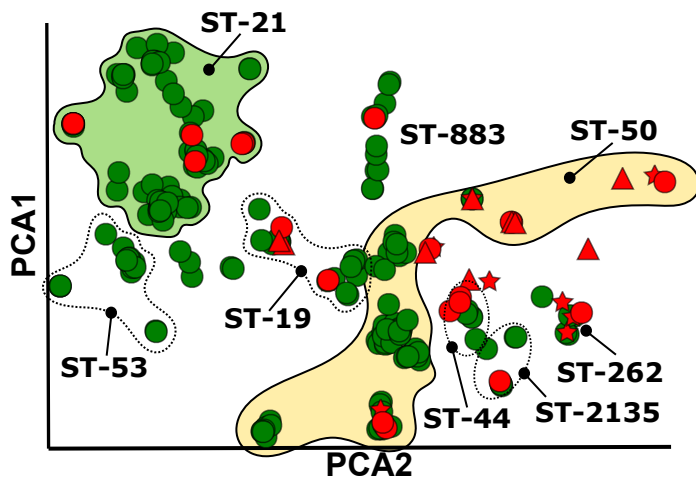
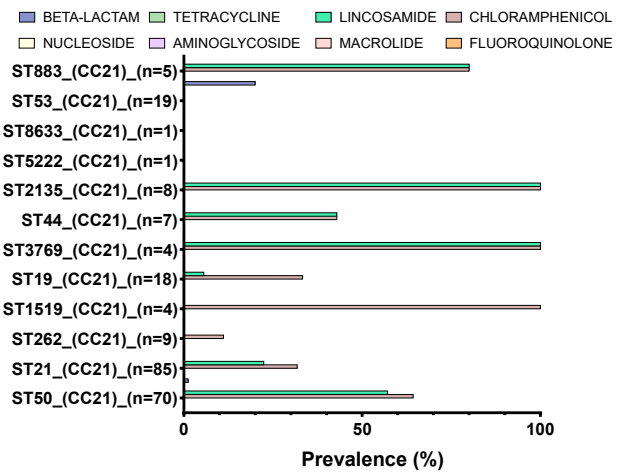
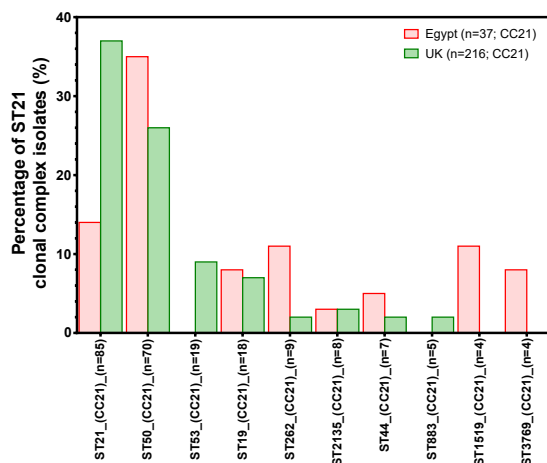
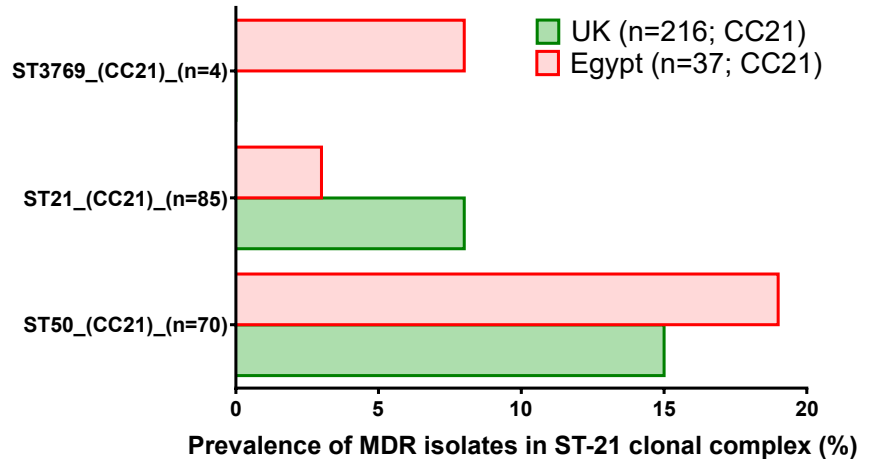


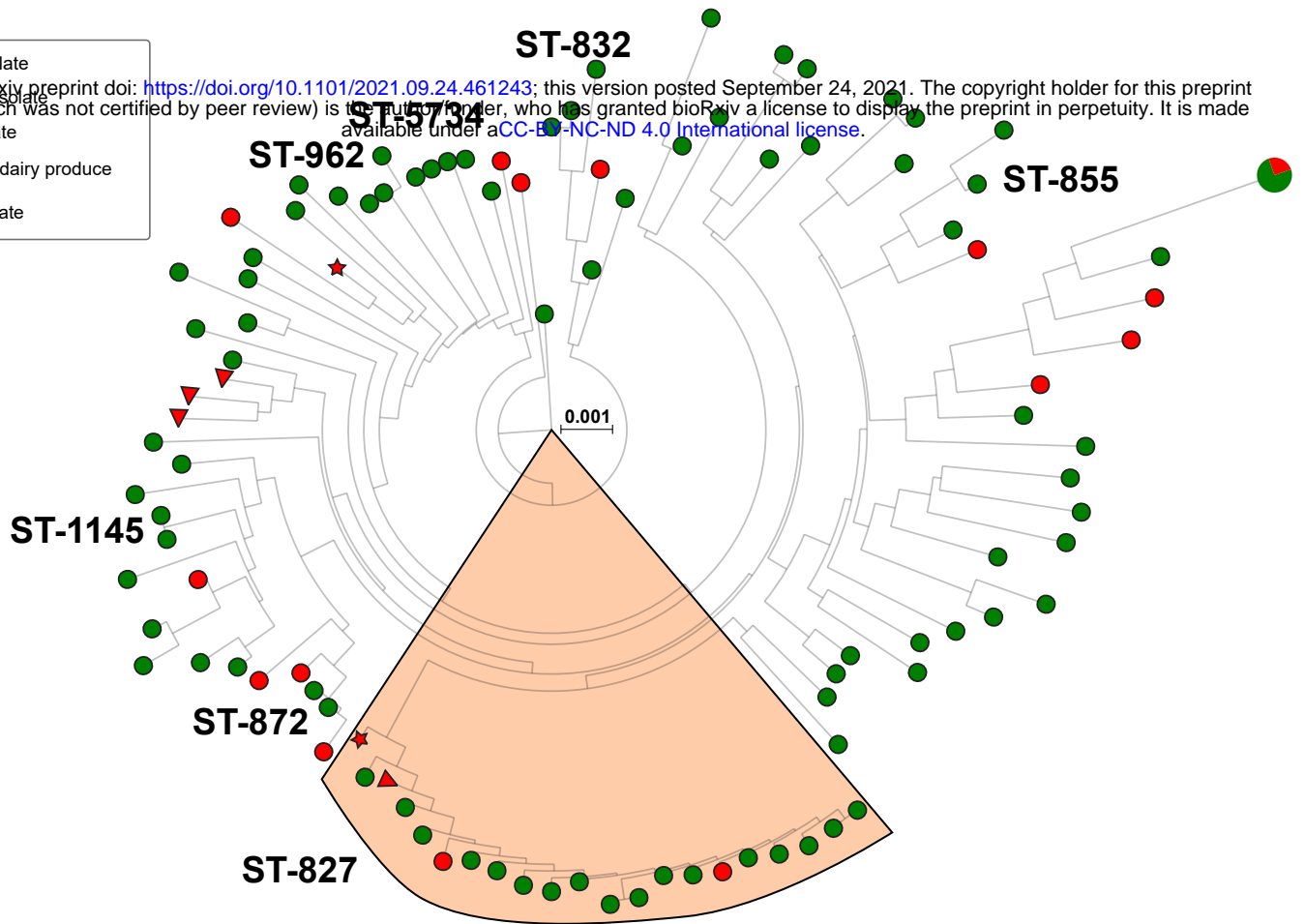
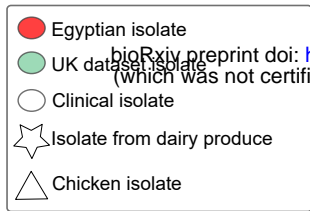
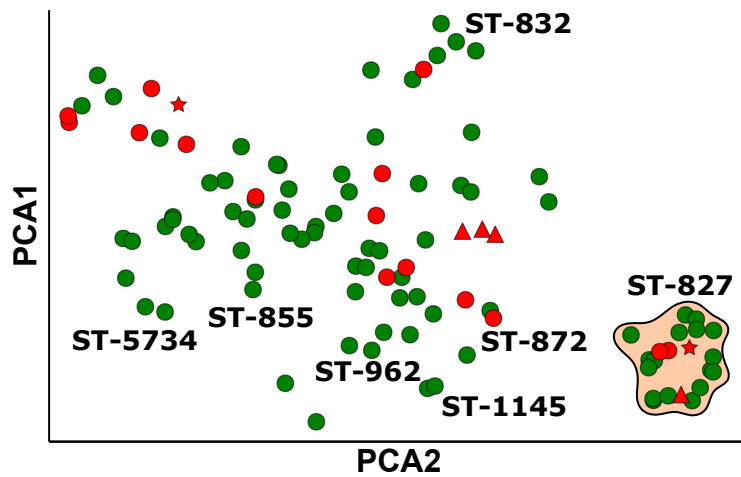
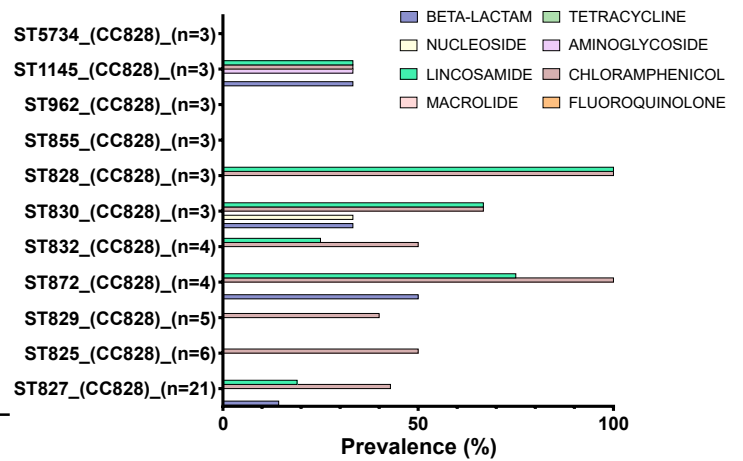
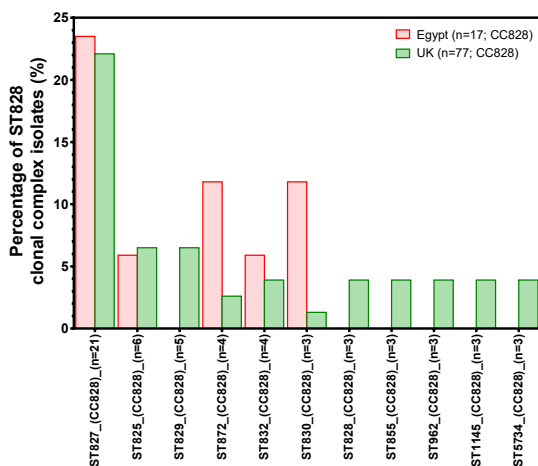
Egyptian isolates (n=112)
Collected 2017-18

Oxford comparison (n=874)
2011-12 (Cody et al; 2012)

Genetic context (n=204)
2017-18 (pubMLST.org)



A**B****D****C****E**

A**B****D****C****E**