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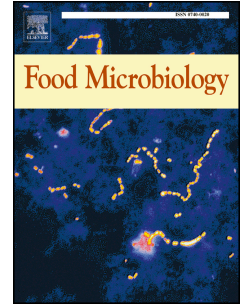
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Stress-resistance associated with multi-host transmission and enhanced biofilm formation at 42°C among hyper-aerotolerant generalist *Campylobacter jejuni*

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1 **Stress-resistance associated with multi-host transmission and**
2 **enhanced biofilm formation at 42°C among hyper-aerotolerant**
3 **generalist *Campylobacter jejuni***

4
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21

37 Abstract

38 One of the emerging conundrums of *Campylobacter* food-borne illness is the bacterial
39 ability to survive stressful environmental conditions. We evaluated the heterogeneity among
40 90 *C. jejuni* and 21 *C. coli* isolates from different sources in Egypt with respect to biofilm
41 formation capabilities (under microaerobic and aerobic atmosphere) and resistance to a
42 range of stressors encountered along the food chain (aerobic stress, refrigeration, freeze-
43 thaw, heat, peracetic acid, and osmotic stress). High prevalence (63%) of hyper-aerotolerant
44 (HAT) isolates was observed, exhibiting also a significantly high tolerance to heat, osmotic
45 stress, refrigeration, and freeze-thaw stress, coupled with high biofilm formation ability
46 which was clearly enhanced under aerobic conditions, suggesting a potential link between
47 stress adaptation and biofilm formation. Most HAT multi-stress resistant and strong biofilm
48 producing *C. jejuni* isolates belonged to host generalist clonal complexes (ST-21, ST-45,
49 ST-48 and ST-206). These findings highlight the potential role of oxidative stress response
50 systems in providing cross-protection (resistance to other multiple stress conditions) and
51 enhancing biofilm formation in *Campylobacter* and suggest that selective pressures encoun-
52 tered in hostile environments have shaped the epidemiology of *C. jejuni* in Egypt by select-
53 ing the transmission of highly adapted isolates, thus promoting the colonization of multiple
54 host species by important disease-causing lineages.

55

56 Keywords: *Campylobacter*, aerotolerance, multi-stress tolerance, generalist lineages, bio-
57 film formation.

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71 1. Introduction

72 *Campylobacter* is the most common causative agent of bacterial gastroenteritis world-
73 wide, with particularly high incidence in low- and middle-income countries (Ruiz-Palacios,
74 2007; Kaakoush *et al.*, 2015). *Campylobacter* is a microaerophilic, fastidious bacterium
75 with an optimal growth temperature range between 37 degrees and 42 degrees Celsius (Ga-
76 rénaux, 2008). *Campylobacter* is part of the intestinal microbiota of a wide range of ani-
77 mals, which can act as reservoirs for zoonotic transmission to humans. Typically, human
78 infection is primarily associated with the consumption of contaminated poultry and meat
79 products, or unpasteurized milk although environmental sources also serve as a transmission
80 route (El-Zamkan and Abdel Hameed, 2016; Newell *et al.*, 2017). Furthermore, transmis-
81 sion of *Campylobacter* can be a result of poor hygiene and/or inadequate food preparation
82 methods in both developed and developing countries (Kennedy *et al.*, 2011; El-Tras *et al.*,
83 2015). In Egypt, campylobacteriosis is a significant public health burden, causing occasional
84 infection in adults, but more frequently in children under the age of 2 years with an inci-
85 dence rate of 1.5 episodes per child-year (Rao *et al.*, 2001; ElGendy *et al.*, 2018).

86 Specific phenotypes appear to be more able to survive and persist under harsh environ-
87 mental conditions, which favor zoonotic transmission and help combat intervention methods
88 employed in poultry processing plants to decrease *Campylobacter* contamination
89 (Bronowski *et al.*, 2014; Yahara *et al.*, 2017). These include the ability to withstand high
90 oxygen tensions, temperature shifts, high osmolarity and physical treatments with hot water,
91 chilling and freezing, and peracetic acid (PAA) (Chen *et al.*, 2014; Umaraw *et al.*, 2017).
92 Convergent evolution of different survival strategies predates our determination to decon-
93 taminant food products, facilitating mechanisms to cope with different stress conditions
94 (Jackson *et al.*, 2009). The ubiquity of *Campylobacter* in the environment challenges our
95 efforts to eliminate this bacterium from the food chain (Omara *et al.*, 2015; Oh *et al.*, 2019),
96 and it is not clear how *Campylobacter* is able to survive the multiple stresses imposed dur-
97 ing food preservation, transportation and cooking (Jong *et al.*, 2012). The ability of this bac-
98 terium to persist and cause gastroenteritis is in contrast to the difficulty of handling it in the
99 laboratory due to its fastidious nature (Garénaux, 2008; Pascoe *et al.*, 2019).

100 Nevertheless, the formation of biofilms and the capability to withstand oxidative stress
101 are among the major strategies used by *Campylobacter* to survive under stressful conditions
102 (Pascoe *et al.*, 2015; Karki *et al.*, 2018). Generally, biofilms are defined as multicellular lay-
103 ers of bacteria embedded within a matrix of extracellular polymeric substances that consist-
104 ed of proteins including enzymes, DNA, RNA, polysaccharides, and water. Bacterial bio-

105 film is considered as a key player in the bacterial survival in diverse ecological niches
106 (Steenackers *et al.*, 2016). Biofilm formation protects bacteria from diverse environmental
107 stressors (Chang *et al.*, 2007). Moreover, bacteria encased in biofilms have been reported to
108 be 1,000-fold more resistant to antimicrobial agents than their planktonic counterparts (Fux
109 *et al.*, 2005), whereas, in human infection, bacteria that produce biofilms are better protected
110 against host defense mechanisms (Ciofu *et al.*, 2015). In food production environments, the
111 presence of *Campylobacter* encased in biofilms formed on food processing surfaces protects
112 it from cleaning and sanitation measures, and facilitates dissemination leading to further
113 contamination of various food products, thus increase its potential to cause disease (Nguyen
114 *et al.*, 2012; Yahara *et al.*, 2017).

115 Characterization of *C. jejuni* genotypes based on multi-locus sequence typing (MLST)
116 from the large, curated online pubMLST database¹ provides evidence of host restricted line-
117 ages, or host specialists, that are predominantly found in only one particular host species
118 (Dingle *et al.*, 2001; Jolley *et al.*, 2018). On the contrary, host-generalist lineages are more
119 promiscuous and can regularly be isolated from multiple host sources, including the globally
120 disseminated clonal complexes (CCs) ST-21, ST-48, ST-206 and ST-45 (Sheppard *et al.*,
121 2010, 2014, 2018). Lineages with broad host ranges are frequently isolated from multiple
122 animal species and are a major cause of human disease (Sheppard *et al.*, 2009a, b; Cody *et al.*,
123 2013). Presumably, host generalist lineages may be better equipped to withstand hostile
124 environmental conditions, but this remains uncharacterized.

125 To fill this important knowledge gap, the survival rates of 111 *Campylobacter* isolates
126 from different sources in Egypt were screened under different stress conditions which mimic
127 ecological niches encountered in farms and food processing industries and which must be
128 overcome for zoonotic transmission to humans.

129

130 2. Materials and Methods

131 2.1. Bacterial isolates and culture conditions

132 A total of 111 *Campylobacter* isolates were collected in Cairo, Egypt, from September
133 2017 to December 2018, including 57 clinical isolates, 24 from dairy products and 30 from
134 broiler carcasses (Supplementary Table S1). Clinical isolates were isolated from stool sam-
135 ples of patients having gastroenteritis admitted to two different hospitals in downtown Cai-
136 ro. A stratified randomized sampling approach was conducted to include *Campylobacter*
137 isolated from food samples from different retail stores located around the study region. The

138 isolation and enumeration of *Campylobacter* isolates from different food matrices was per-
139 formed according to the ISO 10272-1 (Enrichment Method; Detection of *Campylobacter*
140 spp. after Selective Enrichment). All isolates were subcultured from -80°C frozen stocks
141 onto Mueller-Hinton (MH) agar (Oxoid, United Kingdom). Plates were incubated at 42
142 $\pm 1^{\circ}\text{C}$ under microaerophilic conditions using AnaeroGenTM 2.5L sachets (Oxoid, United
143 Kingdom). Genomic DNA was extracted from cultures using the QIAamp DNA Mini Kit
144 (QIAGEN, UK), according to the manufacturer's instructions. DNA was quantified using a
145 Nanodrop spectrophotometer before subsequent genome sequencing.

146 2.2. Genome sequencing

147 *Campylobacter* isolates (n=111) were sequenced using an Illumina MiSeq benchtop se-
148 quencer (Supplementary Table S2 for genome assembly quality features). Libraries were
149 prepared using the Nextera XT Library Preparation Kit according to standard protocols and
150 sequenced using a 2×300 bp paired end v3 reagent kit (Illumina), following the manufac-
151 turer's protocol (Kirk *et al.*, 2018). Raw sequence reads are available on the NCBI and the
152 SRA under BioProject PRJNA576513 ([https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576513)
153 [513](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA576513)). De novo assembly of genomes were done using SPAdes (version 3.8.0) (Bankevich *et*
154 *al.*, 2012) and archived on the BIGSdb web-based database platform (Jolley and Maiden,
155 2010), enabling species identification and MLST. *Campylobacter* MLST genotypes (se-
156 quence types (STs) were assigned based on allele definitions from pubMLST¹ and CCs were
157 defined by the most common sequence type sharing at least five out of seven alleles (Dingle
158 *et al.*, 2001).

159 Phylogenetic trees were reconstructed for the 90 *C. jejuni* and 21 *C. coli*. Core genome
160 MLST (cgMLST) analysis was performed using Genome Profiler software (GeP v1.0.1)
161 (Zhang *et al.*, 2015), using *C. jejuni*_NCTC11168 as reference genome for both *C. jejuni*
162 and *C. coli* analysis (Parkhill *et al.*, 2000). Core genome polymorphic genes (*i.e.* those genes
163 with at least one nucleotide difference among all 90 *C. jejuni* or 21 *C. coli* isolates) were
164 selected for cgMLST phylogenetic tree construction and concatenated in a new fasta file us-
165 ing the [extract_concat_cgMLST_genes.rb](https://github.com/JoseCoboDiaz/concat_cgMLST_genes) ruby script
166 (https://github.com/JoseCoboDiaz/concat_cgMLST_genes). The concatenated gene-by-gene
167 alignments and phylogenetic tree were obtained with MAFFT version 7 (Katoh *et al.*, 2019)
168 using default parameters for alignment and the Neighbor-Joining method, the Jukes-Cantor
169 substitution model and 1000 bootstrap resampling for the construction of the phylogenetic
170 tree. Plots were plotted in using the R-package *ape* to read Newick files.

171 2.3. Stress tolerance of *Campylobacter* isolates.

172 For all the stress tolerance tests, *Campylobacter* isolates were grown on MH agar over-
173 night at 42°C under microaerobic conditions generated using the CampyGen system
174 (CN0035, Oxoid). Afterwards, isolates were harvested and resuspended in fresh MH broth
175 to an optical density at 600 nm (OD₆₀₀) of 0.1 ($\approx 10^8$ colony forming units (CFU) prior to
176 testing their survival to different stressors as described below. Absence of *Campylobacter* in
177 the artificially inoculated chicken skin and milk was assayed by suspension of one gram
178 chicken skin pieces or one ml of milk in 9 ml Bolton selective enrichment broth (Oxoid,
179 United Kingdom), followed by incubation under microaerobic conditions at 41.5°C for 48
180 hours. Afterwards, 100 µL culture was spread on modified charcoal-cefoperazone-
181 deoxycholate agar (mCCDA; Oxoid, United Kingdom) and incubated at 41.5°C for 48
182 hours. All assays included negative control. For all stress tolerance assays, each experiment
183 was performed using three biological replicates with the difference in viable cell counts be-
184 tween replicates was less than 0.5 log₁₀ CFU/mL, all stress tolerance assays results are elu-
185 cidated in Supplementary Table S1.

186

187 2.3.1. Aerotolerance assay

188 The aerotolerance assay was performed as previously described (Oh *et al.*, 2015).
189 Bacterial suspensions adjusted at OD₆₀₀ of 0.1 were incubated aerobically with shak-
190 ing at 200 rpm and 42°C. Aliquots from all bacterial suspensions were taken at 0, 12,
191 and 24 h for serial dilution followed by plating onto Preston *Campylobacter* selec-
192 tive agar (Oxoid, United Kingdom) for bacterial enumeration. *Campylobacter* iso-
193 lates that were not able to survive under aerobic shaking at 200 rpm for 12 h were
194 classified as aero-sensitive (AS), while those that did survive under aerobic shaking
195 at 200 rpm for 12 h were classified as aero-tolerant (AT), and those that survived for
196 more than 24 h under aerobic shaking at 200 rpm were classified as hyper-
197 aerotolerant (HAT). For comparison purposes, a similar experimental design was
198 performed under microaerophilic conditions.

199

200 2.3.2. Survival under refrigerated temperature stress

201 Survival under refrigeration temperature at 4°C was evaluated as previously de-
202 scribed (Oh *et al.*, 2017). In brief, small pieces of raw chicken skin (0.2 gram/piece)
203 that were previously prepared by cutting from chicken thighs using a sterile blade,
204 were placed in 96-well microtiter plate. A total of 100 µL of bacterial suspensions

205 previously adjusted to OD₆₀₀ of 0.1 were used to contaminate the chicken skin sam-
206 ples, followed by incubation of 96-well plates at 4°C. Chicken skin samples were
207 then taken at 0, 1, 3 and 7 days of incubation and transferred to 15 mL falcon tubes
208 containing 1 mL of fresh MH broth, followed by vortexing for 2 minutes and plating
209 of serial dilutions onto Preston *Campylobacter* selective agar for bacterial enumera-
210 tion.

211

212 2.3.3. Survival under chemical decontamination stress

213 Peracetic acid was used to study the survival rates of *Campylobacter* following a
214 chemical decontamination process, as described (Oh *et al.*, 2018). Raw chicken skin
215 pieces prepared as described earlier were spiked with 100 µL of bacterial suspen-
216 sions adjusted to an OD₆₀₀ of 0.1 followed by incubation at 4°C for 1 h under micro-
217 aerobic conditions. Each chicken skin sample was immersed in 750 ppm PAA (Sig-
218 ma Aldrich, St. Louis, MO, USA) for 15 seconds, washed in ultra-pure water and
219 transferred to 15 mL falcon tubes containing 1 mL of fresh MH broth followed by
220 vortexing for 2 minutes and plating of serial dilutions onto Preston *Campylobacter*
221 selective agar for bacterial enumeration.

222

223 2.3.4. Survival following freeze-thaw stress

224 Following the contamination of each chicken skin piece placed into the wells of 96-
225 well microtiter plates with 100 µL of bacterial suspensions adjusted to an OD₆₀₀ of
226 0.1, samples were subjected to freezing at -20°C for 1, 3 and 7 days followed by
227 thawing at 4°C for 2 h. The treated chicken samples were subsequently transferred to
228 15 mL falcon tubes containing 1 mL of fresh MH broth followed by vortexing for 2
229 minutes and plating of serial dilutions onto Preston *Campylobacter* selective agar for
230 bacterial enumeration.

231

232 2.3.5. Survival to heat stress

233 Briefly, 195 µL of whole milk were inoculated with 5 µL aliquots of an overnight
234 culture of *Campylobacter* isolates (OD₆₀₀ =0.1) in 96-well plates. The inoculated
235 plates were then subjected to heat treatment using a thermocycler (Applied Biosys-
236 tems, 2720) at 72°C for 15 and 30 seconds. Aliquots from each bacterial suspension
237 were taken at each time interval for serial dilution and bacterial enumeration on Pres-
238 ton *Campylobacter* selective agar.

239

240 2.3.6. Survival to osmotic stress

241 MH broth supplemented with 0%, 2%, and 4% NaCl (wt/vol) was inoculated with 5
242 μL aliquots of an overnight culture ($\text{OD}_{600} = 0.1$) of the *Campylobacter* isolates in
243 96-well plates. Following overnight incubation at 42°C under microaerobic condi-
244 tions, aliquots from each bacterial suspension were taken for serial dilution and bac-
245 terial enumeration on Preston *Campylobacter* selective agar.

246 2.4. Biofilm formation

247 Crystal violet staining technique was used for the quantification of biofilm formation of
248 *Campylobacter* isolates as previously described (Pascoe *et al.*, 2015). *Campylobacter* iso-
249 lates were inoculated into MH broth with OD_{600} adjusted to 1. Then, 5 μL aliquots of the
250 bacterial suspensions were used to inoculate 195 μL of liquid MH media in 96-well plates
251 (Thermo Fisher Scientific, Hudson, NH, USA). Following incubation of the plates under
252 microaerobic conditions with shaking at 42°C for 48 h in a sealed container (to prevent
253 sample evaporation), the culture media was gently removed, and the wells were washed
254 with phosphate-buffered saline. Fixation of the bacteria adhered to the wells was performed
255 by adding 200 μL of Bouin's solution (7.5 mL picric acid; 2.5 mL 40% formaldehyde; 0.5
256 mL acetic acid), incubating for 15 minutes followed by washing 3x with PBS. Plates were
257 left to air dry in an inverted position and the adhered bacteria were stained using 200 μL of a
258 0.1% (w/v) crystal violet solution for 5 minutes. Extraction of crystal violet from the ad-
259 hered bacteria was performed by adding 200 μL of an 80:20 (vol/vol) ethanol/acetone solu-
260 tion, followed by incubation for 10 minutes. The OD_{600} value of the extracted crystal violet
261 was measured using a microplate ELISA reader. These OD_{600} readings were used to quanti-
262 fy the bacterial adherence to the surface and the ability of the isolates to form biofilms. The
263 whole procedure was also repeated under aerobiosis. The isolates were classified according
264 to their OD_{600} score into strong biofilm producers (SBPs) with OD_{600} values above 0.272,
265 moderate biofilm producers (MBPs) with OD_{600} values between 0.201 and 0.272, and weak
266 biofilm producers (WBPs) with OD_{600} values below 0.201. Broth cultures with no bacterial
267 inoculation and stained using the same method were used as a negative control and the val-
268 ues obtained were subtracted for background correction. To assure the reproducibility of the
269 results, each isolate was tested for its biofilm formation in triplicate.

270

271 2.5. Statistical analysis

272 Statistical analysis included the comparison of different isolate groups based on their bio-
273 film formation ability and stress resistance profile at the various time points (for aerophilic
274 and microaerophilic growth and survival under refrigeration, freeze-thaw and heat stress) or
275 stressors concentrations (PAA and NaCl stress) tested. Log₁₀ CFU/mL values from the
276 stress tolerance assays were compared using Wilcoxon test with the R-package *stats* v3.6.2.
277 Plots were performed using the R-packages *ggplot2* and *ggpubr*, and statistical results were
278 added with the *stat_compare_means* command within the *ggplot* function.

279

280 3. Results

281 3.1. High occurrence of hyper-aerotolerance among *Campylobacter* isolates

282 In total, 111 *Campylobacter* isolates from three different sources were screened for their
283 aerotolerance (Figure 1A; Supplementary Figure S1A). All *Campylobacter* isolates grew
284 well under microaerobic conditions with a steady increase in viable counts of up to 2 log₁₀
285 CFU/mL regardless of the species or source of isolation (Figure 1B).

286 A high prevalence of HAT isolates was observed (63%, 70/111). Under aerobic incuba-
287 tion, HAT strains remained viable after a 24 h with a final mean concentration of 3.25 ±2.7
288 log₁₀ CFU/mL, whereas 22.5% (25/111) of the isolates were AT and maintained their viabil-
289 ity for 12 h with a final mean concentration of 2.6 ±1.4 log₁₀ CFU/mL, and 14.5% (16/111)
290 of the tested isolates were AS and lost viability before 12 h (Figure 1A).

291 When analyzing the aerotolerance of the isolates by their isolation source, it was ob-
292 served that HAT isolates were equally recovered from the three different isolation sources,
293 the prevalence of HAT isolates being 66.6% (38/57) of clinical samples, 62.5% (15/24) of
294 dairy products and 56.6% (17/30) of broiler carcasses isolates (Supplementary Figure S1).

295

296 3.2. Association of high aerobic tolerance with survival under multiple stress conditions in 297 *Campylobacter* isolates

298 Storage at refrigeration temperature (4°C) for 7 days instigated a significant decrease in
299 CFU/mL in AS and AT isolates compared to HAT isolates ($p \leq 0.01$) (Figure 1D). Half of
300 HAT isolates (50%, 35/70) exhibited an enhanced tolerance to refrigeration temperature,
301 reaching a final concentration of 1.5 ±1 log₁₀ CFU/mL after 7 days, while only two AT iso-
302 lates and none of the AS strains survived at this sampling point. Similarly, both AT and AS
303 isolates were significantly more sensitive to freeze-thaw stress than HAT isolates ($p \leq 0.001$)
304 (Figure 1E). Thus, while 60% of HAT isolates (42/70) survived at -20 °C on day 7, reach-

305 ing a final mean concentration of $2.6 \pm 2 \log_{10}$ CFU/mL, only three AT isolates (12%; 3/25)
306 survived with a final mean concentration of $1.3 \pm 0.3 \log_{10}$ CFU/mL, and no survivors were
307 obtained for AS isolates.

308 Consistent with the data of refrigeration and freeze-thaw tolerance tests, the aerotoler-
309 ance status was significantly associated with tolerance to heat stress ($p \leq 0.01$) (Figure 1F).
310 While the 60% of HAT isolates (42/70) survived upon exposure to 70°C for 30 sec, reaching
311 a final mean concentration of $3.2 \pm 2 \log_{10}$ CFU/mL, only four AT isolates (16%; 4/25) and
312 two AS isolates (15.5%; 2/16) survived to such a stress, reaching a final mean concentration
313 of 2.9 ± 1.7 and $1.8 \pm 1.5 \log_{10}$ CFU/mL, respectively.

314 Finally, HAT isolates also had a significantly higher tolerance to hyperosmotic stress at
315 4% NaCl than AT and AS isolates ($p \leq 0.0001$) (Figure 1H). In fact, 58% (41/70) of HAT
316 isolates survived hyperosmolarity, with a final mean concentration of $2.7 \pm 1.8 \log_{10}$
317 CFU/mL, while only two AT isolates (8%; 2/25) were able to survive reaching a final mean
318 concentration of $2.2 \pm 0.3 \log_{10}$ CFU/mL and none of the AS isolates survived 2% or 4%
319 NaCl exposure.

320

321 3.3. Higher tolerance of HAT and AT *Campylobacter* isolates to peracetic acid

322 Exposure to PAA equally reduced the viability of HAT and AT isolates by $\sim 4.5 \log_{10}$
323 CFU/mL. The initial mean concentrations of HAT and AT isolates, 7.3 ± 1.1 and 7.4 ± 1.2
324 \log_{10} CFU/mL, declined after treatment to a final viable count of 2.9 ± 1.8 and $2.6 \pm 1.5 \log_{10}$
325 CFU/mL, respectively. Based on these observations, no significant difference was revealed
326 between HAT and AT isolates, while none of the AS isolates survived upon exposure to
327 PAA (Figure 1G).

328

329 3.4. Differences in stress tolerance between *C. jejuni* and *C. coli*

330 *C. jejuni* was significantly more tolerant to aerobic stress than *C. coli*, showing a higher
331 survival potential at 12 h and 24 h ($p < 0.01$) (Figure 1A), despite the fact that 52% (11/21)
332 of *C. coli* isolates were HAT, with a final mean concentration of $2.2 \pm 1.7 \log_{10}$ CFU/mL af-
333 ter a 24 h of aerobic incubation. Yet, HAT *C. jejuni* isolates were more abundant among the
334 collection (65.5%, 59/90) (Figure 3C), reaching a final mean concentration $3.3 \pm 2.6 \log_{10}$
335 CFU/mL after a 24 h of aerobic incubation.

336 Likewise, despite the clear reduction in CFU/mL observed for both species following ex-
337 posure to PAA, the majority of *C. jejuni* isolates (80%, 72/90) exhibited a significantly

338 higher resistance to PAA than *C. coli* ($p \leq 0.05$) (Figure 1G), reaching a final mean concen-
 339 tration of $2.9 \pm 1.8 \log_{10}$ CFU/mL. Remarkably, none of the *C. coli* isolates survived upon
 340 exposure to 4% NaCl, while half of *C. jejuni* isolates survived with final mean concentration
 341 of $2.75 \pm 1.8 \log_{10}$ CFU/mL, and such a difference between species was significant ($p \leq$
 342 0.001) (Figure 1H).

343 On the contrary, no significant differences ($p > 0.05$) were detected between the two spe-
 344 cies in their tolerance to refrigeration, freeze-thaw or heat stresses (Figure 1D, E, F), with
 345 isolates from both species displaying nearly the same final mean concentrations upon expo-
 346 sure to 7 days of refrigeration or freezing, and 30 sec at 70°C, *i.e.* 1.5 ± 1 , 2.6 ± 2.1 , and 3.2
 347 $\pm 2.15 \log_{10}$ CFU/mL for *C. jejuni* and 1.4 ± 0.45 , 1.8 ± 1.15 , $3.2 \pm 1.7 \log_{10}$ CFU/mL for *C.*
 348 *coli*, respectively.

349

350 3.5. Clonal population structure and its association with stress-resistance among *C. jejuni* 351 isolates

352 To evaluate whether *C. jejuni* host generalist lineages might exhibit a higher potential to
 353 survive under various stressful conditions, providing them a beneficial advantage, at least in
 354 part, for transmission between multiple hosts during bacterial transmission, the population
 355 structure of *C. jejuni* was characterized using MLST and a phylogenetic tree was construct-
 356 ed using concatenated gene-by-gene alignments of polymorphic core genes, isolates were
 357 grouped into two distinct clusters according to their species with *C. jejuni* being more di-
 358 verse than *C. coli* in regards to their CCs distributions (Figure 3A, B).

359 The MLST analyses revealed that the 90 *C. jejuni* isolates were classified into 15 differ-
 360 ent CCs, while 9 were not assigned to known CCs (Supplementary Table S1). *C.*
 361 *jejuni* isolates were classified as ``host specialist`` isolates, belonging to ST□257 ($n=4$),
 362 ST□464 ($n=7$), ST□353 ($n=3$), ST□354 ($n=3$), ST□460 ($n=2$), ST□1034 ($n=2$),
 363 ST□1287 ($n=2$), ST□42 ($n=1$), ST□573 ($n=1$), ST□574 ($n=1$) and ST□658 ($n=1$)
 364 CCs, and ``host generalist`` isolates, belonging to ST□21 ($n=36$), ST-48 ($n=7$), ST-45
 365 ($n=1$) and ST-206 ($n=10$) CCs (Sheppard *et al.*, 2014). Among the isolated *C. jejuni*, host
 366 generalist isolates were more dominant (60%, 54/90) (Figure 3B).

367

368 3.5.1. Host generalist *C. jejuni* lineages showed an enhanced capability to respond to 369 oxidative stress

370 The majority of host generalist isolates were found to be HAT (94.5%, 51/54) and
371 survived significantly better ($p \leq 0.0001$) after 24 h of incubation under aerobic atmosphere,
372 with a final mean concentration of $3.8 \pm 2.1 \log_{10}$ CFU/mL, than isolates from other lineages,
373 which barely survived the aerobic stress and showed a drastic decline in their population,
374 reaching final mean viable counts of $1.6 \pm 1 \log_{10}$ CFU/mL (Figure 2A).

375

376 3.5.2. Multi-stress tolerance among host generalist *C. jejuni* lineages

377 Isolates assigned to generalist lineages showed a significantly higher tolerance to all five
378 stress conditions (*i.e.* refrigeration, freeze-thaw, heat, PAA and NaCl) than isolates of host
379 specialist lineages, which experienced a drastic reduction in survival under the stress condi-
380 tions tested (Figure 2D, E, F, G, H).

381 With regards to refrigeration and freeze-thaw stresses, the greatest decline was observed
382 among the isolates assigned to host specialist lineages on day 7, with the survival of only
383 two isolates (final mean concentration of $1.4 \pm 0.3 \log_{10}$ CFU/mL) and three isolates (final
384 mean concentration of $1.13 \pm 0.2 \log_{10}$ CFU/mL), respectively; whereas 59% (32/54) and
385 70% (38/54) of generalist isolates survived for 7 days under refrigeration or freeze-thaw
386 stresses, with a final mean concentration of $1.08 \pm 1.5 \log_{10}$ CFU/mL and $2.1 \pm 2.6 \log_{10}$
387 CFU/mL, respectively ($p \leq 0.0001$) (Figure 2D, E).

388 Similarly, the majority of isolates assigned to host generalist lineages (68.5%, 37/54)
389 survived heating for 30 sec to reach a final mean concentration of $3.2 \pm 2 \log_{10}$ CFU/mL,
390 while a low percentage (16.6%, 6/36) of other lineages survived this heat stress, with a final
391 mean concentration of $2.9 \pm 1.7 \log_{10}$ CFU/mL (Figure 2F). Likewise, host generalist isolates
392 efficiently survived upon exposure to PAA and NaCl, with survival rates being significantly
393 higher than those of host specialist *C. jejuni* lineages isolates ($p \leq 0.001$ and $p \leq 0.0001$, re-
394 spectively) (Figure 2G, H). All isolates assigned to host generalist CCs survived upon expo-
395 sure to 750 ppm PAA with mean viable count of $2.9 \pm 1.8 \log_{10}$ CFU/mL, while 52%
396 (19/36) of the host specialist isolates were able to survive, with a mean final concentration
397 of $2.9 \pm 1.8 \log_{10}$ CFU/mL (Figure 2G). Regarding NaCl stress, 74% (40/54) of the host gen-
398 eralist isolates survived the 4% NaCl exposure, with a final mean concentration of 2.7 ± 1.8
399 \log_{10} CFU/mL, while only two host specialist isolates (5.5%, 2/36) survived similar NaCl
400 concentration, with mean viable cell counts of $2.6 \pm 0.6 \log_{10}$ CFU/mL (Figure 2H).

401

402

403 3.6. Exposure to aerobic stress enhances biofilm formation in *Campylobacter*

404 Initially, when evaluating the differences between biofilm formation potential of *C. jejuni*
405 and *C. coli* isolates under microaerobic conditions, both species showed similar capability of
406 biofilm formation with a mean OD₆₀₀ of 0.4 ±0.3 and 0.24 ±0.13, respectively, whereas un-
407 der aerobic conditions the biofilm formation potential was enhanced with no significance
408 differences being observed either between *C. jejuni* and *C. coli*, with mean OD₆₀₀ of 0.5
409 ±0.36 and 0.4 ±0.25, respectively ($p > 0.05$) (Figure 1C). Interestingly, under microaerobic
410 conditions 38.8% (35/90) of *C. jejuni* isolates were classified as SBPs, while under aerobic
411 conditions this percentage increased to reach 78.8% (71/90). On the other hand, the preva-
412 lence of SBP isolates for *C. coli* increased from a 19% (4/21) under microaerobic conditions
413 to 71.4% (15/21) under aerobic stress (Figure 3).

414 In relation to the association between aerotolerance and biofilm formation potential, it
415 was observed that *Campylobacter* displayed variable biofilm formation capabilities depend-
416 ing on aerotolerance status, with HAT isolates exhibiting a significantly higher adhesion
417 capability and biofilm formation potential compared to AT and AS isolates ($p \leq 0.0001$)
418 (Figure 1C).

419 Under microaerobic conditions, more than half (52.8%, 37/70) of the HAT isolates were
420 SBPs, with a mean OD₆₀₀ of 0.49 ±0.2, while 34.2% (24/70) were MBPs, with a mean
421 OD₆₀₀ of 0.23 ±0.02. Remarkably, a significant change in biofilm formation ability was ob-
422 served among the majority of HAT isolates under aerobic stress, where 95.7% (67/70) of
423 HAT isolates were SBPs, with a mean OD₆₀₀ of 0.58 ±0.3 (Figure 3). Also, 76% (19/25) of
424 AT isolates were found to be SBPs under aerobic stress, with a mean OD₆₀₀ of 0.53 ±0.2
425 compared to a percentage of 8% (2/25) under microaerobic conditions. Interestingly, while
426 AS isolates were all classified as WBPs under microaerobic conditions, with a mean OD₆₀₀
427 of 0.14 ±0.05, an enhancement in biofilm formation potential was also observed under aero-
428 bic stress, with 56.2% (9/16) isolates being classified as MBPs (Figure 3).

429

430 3.6.1. Clonal lineages differ in terms of their biofilm forming capabilities

431 Biofilm formation ability was not equally distributed among *C. jejuni* lineages. When
432 exposed to microaerophilic conditions, host generalist isolates displayed a significantly
433 higher potential for biofilm formation (mean OD₆₀₀ of 0.4 ±0.28) compared to isolates of
434 other lineages (mean OD₆₀₀ of 0.18 ±0.09) ($p \leq 0.0001$) (Figure 2C). Under favorable micro-
435 aerobic conditions, 61% of the generalist isolates were SBPs, compared to only 5.5% for
436 isolates of other lineages. Upon exposure to oxygen, *in vitro* biofilm production abilities of

437 both generalist and specialist lineages increased (Figure 2C). Still, the potential of host gen-
438 eralist isolates to form biofilms was significantly higher (mean OD₆₀₀ of 0.54 ±0.3) than that
439 of isolates from other lineages (mean OD₆₀₀ of 0.3 ±0.16) ($p \leq 0.0001$) (Figure 2C), with
440 98% of the generalists being classified as SBPs and none of them as WBPs, while 50% of
441 isolates from other lineages were classified as SBPs, 27.8% as MBPs, and 22.2% as WBPs.
442 Based on these results, HAT isolates that were assigned to host generalist CCs had the
443 strongest biofilm formation potential under microaerobic “favorable” conditions, which was
444 enhanced under aerobic conditions (Figure 3B, C).

445

446 4. Discussion

447 During transmission, bacteria must tolerate suboptimal conditions to successfully
448 establish an infection in the new host (Begley and Hill, 2015). *Campylobacter* are fastidious
449 organisms that are particularly sensitive to environmental stresses and that require
450 microaerophilic (5% O₂), capnophilic (10% CO₂), and thermophilic (40-42°C) settings
451 under laboratory conditions (Garénaux, 2008). It is likely that their routes of transmission to
452 humans through the environment, farm, and wild animals may interact in very complex
453 ways (Bronowski *et al.*, 2014).

454 Despite the absence of many classic stress response strategies in *Campylobacter* com-
455 pared to other enteric bacteria, such as the RpoS-mediated stress resistance system, the os-
456 motic shock regulatory system BetAB and some oxidative stress response regulatory ele-
457 ments such as *oxyR* and *soxR*, and *soxS* (Murphy *et al.*, 2006), *Campylobacter* has been iso-
458 lated from environmental sources where neither the atmosphere nor the temperature were
459 optimal for its survival (Chan *et al.*, 2001; Trigui *et al.*, 2015). *Campylobacter* has devel-
460 oped several strategies to survive in a wide range of environmental stressors outside the host
461 and/or during food processing, which likely involve the entry into a viable but non cultura-
462 ble state (Jackson *et al.*, 2009), biofilm formation and lineage-specific variations in stress
463 tolerance (Pascoe *et al.*, 2015; Yahara *et al.*, 2017). The stress response of *C. jejuni* has been
464 previously studied and several regulatory systems have been described, including those me-
465 diating the global “SpoT-dependent stringent response”, which adjusts gene expression
466 pathways to permit survival under a wide range of hostile conditions (Gaynor *et al.*, 2005),
467 and the sigma factor RpoN, which plays a significant role in the resistance of *C. jejuni*
468 against osmotic stress (0.8% NaCl) and acidic pH (Hwang *et al.*, 2011).

469 These resistance strategies can provide the means for survival and transmission of *Cam-*
470 *pylobacter* between different animal reservoirs and hosts. To maintain food quality and en-
471 sure food safety by reducing spoilage and pathogenic bacteria on food products, various in-
472 tervention methods are employed, e.g. heating, modified atmosphere packaging, low storage
473 temperatures, or use of antimicrobials and disinfectants. Nonetheless, whether the interme-
474 diate habitat (particularly from farm to fork) of *Campylobacter* plays an important role in
475 shaping the epidemiology of this food-borne pathogen through selecting well-adapted iso-
476 lates remains unclear. Therefore, this study highlights the role of cross-protection and bio-
477 film formation on *Campylobacter* survival and considers the potential of stress-associated
478 resistance mechanisms for selecting highly adapted *Campylobacter* isolates that can infect
479 multiple hosts.

480 Despite the common perception that *Campylobacter* is sensitive to oxygen, this study re-
481 vealed that large number of isolates were capable of surviving for up to 24 h even under the
482 most hostile atmospheric conditions. With 63% of *Campylobacter* isolates, recovered from
483 the three different isolation sources, were found to be HAT reaching final viable count of
484 $3.25 \pm 2.7 \log_{10}$ CFU/mL after 24 h of aerobic incubation. The greater prevalence of HAT
485 isolates was observed among *C. jejuni* compared to *C. coli* isolates suggests a high capabil-
486 ity of *Campylobacter* isolates, particularly *C. jejuni*, to tolerate oxidative stress. In agree-
487 ment with this finding, several studies have reported the isolation of aerotolerant *Campylo-*
488 *bacter* (Oh *et al.*, 2015; O’Kane and Connerton, 2017). However, in the study conducted by
489 Oh *et al.* (Oh *et al.*, 2015) aerotolerance was evaluated among 70 *C. jejuni* isolates among
490 which only 35.7% were classified as HAT, while another study reported a higher prevalence
491 of hyper-aerotolerance among *C. coli* than among *C. jejuni* isolates (Karki *et al.*, 2018),
492 which is opposing to the results of the present study. Yet, in these previous studies no iso-
493 lates from human stool and dairy products were included. Thus, to the best of our
494 knowledge, this is the first study documenting such a prevalence of HAT *Campylobacter*
495 isolates from three different sources.

496 Despite being thermophilic and ceasing its growth abruptly at temperatures below 30°C
497 (Hazeleger *et al.*, 1998), more than half of the tested HAT *Campylobacter* isolates showed
498 physiological activity and survived for seven days under refrigeration or freeze-thaw stress.
499 Although *Campylobacter* does not possess genes encoding cold shock proteins (Hazeleger
500 *et al.*, 1998), unlike other foodborne pathogens (Horton *et al.*, 2000), this observation sug-
501 gests that some of the *Campylobacter* isolates or lineages tested may harbor other tolerance
502 mechanisms to respond to cold shocks. This observation is in agreement with previous stud-

503 ies (Oh *et al.*, 2018, 2019). However, these previous investigations were focused on *C. je-*
504 *juni* only, unlike the current study which embraces both *C. jejuni* and *C. coli*. From another
505 standpoint, previous studies with *C. coli* have reported its high sensitivity to freeze-thaw
506 stress (Stead and Park, 2000), unlike the present study, where both *C. jejuni* and *C. coli*
507 showed similar tolerance to such a stress. HAT isolates were also significantly more tolerant
508 to heat stress than AT and AS isolates, which disagrees with a recent study (Oh *et al.*, 2019)
509 reporting that HAT isolates displayed sensitivity to heat stress and no significant association
510 of aerotolerance with heat resistance.

511 Since PAA is an effective biocide used for reducing *Campylobacter* populations (Chen *et*
512 *al.*, 2014) and thus on examining its effect on the survival rates of the tested isolates, it was
513 observed that both HAT and AT isolates exhibited an enhanced tolerance to PAA. A pro-
514 posed explanation for such an association is that since PAA is known to decompose to H₂O₂
515 and acetic acid (Yuan *et al.*, 1997), therefore the high tolerance of HAT and AT isolates
516 might be attributed to the increased oxidative stress defense, as previously elucidated (Oh *et*
517 *al.*, 2019).

518 *Campylobacter* is known to be sensitive to hyperosmotic stress (Park, 2002). Indeed, this
519 bacterial pathogen can be inhibited at >2% NaCl (Doyle and Roman, 1982), a food pre-
520 servative commonly used to prevent the growth of foodborne pathogens (Doyle and Glass,
521 2010). The tolerance to hyperosmotic stress was also related to hyperaerotolerance in the
522 present study, with HAT isolates, entirely consisting of *C. jejuni*, showed a significant high
523 tolerance to 4% NaCl exposure. This observation suggests that oxidative stress defense sys-
524 tems may provide cross-protection against osmotic stress, and vice versa, which agrees with
525 a previous study reporting that exposure to 1% NaCl moderately upregulates genes associat-
526 ed with oxidative stress response in *Campylobacter* (Cameron *et al.*, 2012). However, no
527 association was documented in a similar recent study, in which the level of hyper-
528 osmotolerance was variable depending on the isolate itself rather than the aerotolerance po-
529 tential (Oh *et al.*, 2019).

530 The high prevalence of HAT *Campylobacter* isolates in the current study might be a con-
531 tributing factor, at least in part, to the abundance of *Campylobacter* in diverse animal, hu-
532 man, and environmental reservoirs in Egypt (Omara *et al.*, 2015; ElGendy *et al.*, 2018).
533 Moreover, it is apparent that the increased aerotolerance is coupled with a tolerance to tem-
534 perature shifts and high osmolarity, and a possible augmented action of oxidative stress de-
535 fense enzymes decomposing PAA, which will give raise to increase transmission of multi-
536 stress tolerant phenotypes.

537 It has been reported that microenvironments created within biofilms permit *Campylobac-*
538 *ter* survival for long period of time under aerobic atmosphere conditions, providing physical
539 protection of cells from oxygen inactivation (Joshua, 2006). Indeed, in this study, biofilm
540 formation by *Campylobacter* significantly increased under aerobic conditions, with no sig-
541 nificant differences in biofilm formation potential between *C. jejuni* and *C. coli*, contradict-
542 ing previous reports stating that *C. coli* forms less biofilms on inert surfaces than *C. jejuni*
543 (Sulaeman *et al.*, 2010). In addition, 95.7% of HAT *Campylobacter* isolates were found to
544 be SBPs under aerobic conditions and developed biofilms more efficiently than AT and AS
545 isolates. Thus, signifying that *Campylobacter* can thrive in hostile environments in biofilms
546 and highlights the role of oxidative stress as one of the signals that induce biofilm formation
547 in *Campylobacter*, therefore contributing to its dissemination and persistence in poultry
548 houses and slaughter facilities.

549 In accordance with the current study that revealed isolates from generalist lineages (CC-
550 21, CC-45, CC- 48, CC-206) showing a substantial tolerance to temperature variation,
551 disinfectant and high osmotic pressure, a recent genome wide association study demonstrat-
552 ed that some major *C. jejuni* lineages (CC-21, CC-45) possess certain genetic determinants
553 of fitness, associated with tolerance to various pressures encountered through the poultry
554 processing chain (Yahara *et al.*, 2017). Furthermore, isolates of generalist lineages exhibited
555 hyper-aerotolerance potential compared to isolates from other lineages. These findings are
556 in agreement with a previous study (Oh *et al.*, 2015) highlighting the enhanced aerotoler-
557 ance potential of CC-21 and CC-45, which are known to be the major CCs implicated in
558 human gastroenteritis (Nielsen *et al.*, 2010; Colles and Maiden, 2012), while recently CC-48
559 isolates showing hyper-aerotolerance have been reported (Kiatsomphob *et al.*, 2019). None-
560 theless, and to the best of our knowledge, the aerotolerance potential of isolates from ST-
561 206 CC has not been reported before.

562 The enhanced aerotolerance exhibited by generalist *C. jejuni* lineages in the current study
563 might impact their potential for multi-host and foodborne transmission, which is further
564 augmented by the high ability to form biofilms, enabling their survival outside the host and
565 promoting their spread, as previously hypothesized (Woolhouse *et al.*, 2001). Indeed, in the
566 present study, host generalist *C. jejuni* isolates had a higher biofilm formation potential and
567 produced more dense biofilms in oxygen-rich conditions than in oxygen-limited conditions,
568 with 61% and 98% of generalist *C. jejuni* isolates were SBPs under microaerobic and aero-
569 bic conditions, respectively. Such an observation is consistent with a previous study show-

570 ing that production of biofilm increases the protection from aerobic stress in host general-
571 ist *C. jejuni* more than in other host specialist lineages (Pascoe *et al.*, 2015).

572 In conclusion, the results of this study provide an evidence that stress-adapted *Campylo-*
573 *bacter* lineages can thrive better under various environmental stresses than other non-
574 adapted lineages, whereas oxidative-stress defense responses were shown to be associated
575 with enhanced biofilm formation capabilities and with tolerance to various other stress con-
576 ditions, likely due to “cross- protection” mechanisms. Our results show dominance of multi-
577 stress resistant generalist isolates, which, together with the low biosecurity and presence of
578 backyard farming and small-scale livestock production in Egypt (El-Tras *et al.*, 2015), can
579 provide opportunities for the multi-host spread of robust *Campylobacter* lineages. More im-
580 portantly, they suggest that selective pressures encountered in hostile environments prevail-
581 ing throughout food processing have shaped the epidemiology of *C. jejuni* in Egypt by se-
582 lecting the transmission of highly adapted isolates, thus promoting the colonization of mul-
583 tiple host species by important disease-causing lineages and their transmission to humans
584 (Sheppard *et al.*, 2009b; Yahara *et al.*, 2017). Further studies will be crucial to elucidate the
585 molecular determinants and assessing the transcriptome involved in stress or osmoregulation
586 among HAT, AT, and AS isolates which can subsequently lead to establishing efficient
587 measures to control the risk of *Campylobacter* in the food chain.

588

589 Abbreviation

590 Aero-Sensitive (AS)

591 Aero-Tolerant (AT)

592 Clonal Complexes (CCs)

593 Core genome Multilocus Sequence Typing (cgMLST)

594 Hyper-Aerotolerant (HAT)

595 Moderate Biofilm Producers (MBPs)

596 Mueller-Hinton (MH)

597 Multi-Locus Sequence Typing (MLST)

598 Optical Density at 600 nm (OD600)

599 Peracetic Acid (PAA)

600 Sequence Types (STs)

601 Strong Biofilm Producers (SBPs)

602 Weak Biofilm Producers (WBPs)

603

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607

608 Author Contributions

609 M.E., A.A.O. and S.K.S. designed the study and performed the phenotypic studies

610 S.F.M. wrote the paper with some additional edits from M.E and A.A.O.

611 J.K.C. and B.P. sequenced and assembled the genomes
 612 S.F.M. and J.F.C. performed genomic data analysis.
 613 J.F.C performed statistical analysis and phylogenetic tree construction with some additional
 614 contribution from A.M.
 615 M.E. contributed to the acquisition of samples.
 616 All authors contributed and approved the final manuscript.

617
 618 Conflict of Interest

619 All authors declare no conflict of interest.

620
 621 Footnotes

622 1. <https://pubmlst.org/organisms/campylobacter-jejunicoli/>

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858 Figure and Table legends

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860 Figure 1. Survival under stress conditions and biofilm formation ability of 70 HAT, 25 AT
861 and 16 AS *Campylobacter* isolates. (A) Behavior under aerobic stress; (B) Growth in
862 microaerobic conditions; (C) Biofilm formation under aerobic and microaerobic conditions;
863 Tolerance to (D) refrigeration, (E) freeze-thaw, (F) heat, (G) PAA, and (H) NaCl stresses.
864 Statistical significance was determined using the Wilcoxon test (ns: $p > 0.05$, $*p \leq 0.05$, $**p$
865 ≤ 0.01 , $***p \leq 0.001$, $****p \leq 0.0001$).

866

867 Figure 1. Survival under stress conditions and biofilm formation ability of 90 *C. jejuni* and
868 21 *C. coli* isolates. (A) Behavior under aerobic stress; (B) Growth in microaerobic condi-
869 tions; (C) Biofilm formation under aerobic and microaerobic conditions; Tolerance to (D)
870 refrigeration, (E) freeze-thaw, (F) heat, (G) PAA, and (H) NaCl stresses. Statistical signifi-
871 cance was determined using the Wilcoxon test (ns: $p > 0.05$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq$
872 0.001 , $****p \leq 0.0001$).

873

874 Figure 2. Survival under stress conditions and biofilm formation ability of host generalist
875 lineages ($n=54$) and other lineages ($n=36$) of *C. jejuni*. (A) Behavior under aerobic stress;
876 (B) Growth in microaerobic conditions; (C) Biofilm formation under aerobic and
877 microaerobic conditions; Tolerance to (D) refrigeration, (E) freeze-thaw, (F) heat, (G) PAA,
878 and (H) NaCl stresses. Statistical significance was determined using the Wilcoxon test (ns: p
879 > 0.05 , $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$).

880

881 Figure 3. Phylogenetic trees for (A) 21 *C. coli* isolates and (B) 90 *C. jejuni* isolates, using
882 concatenated gene-by-gene alignments of polymorphic core genes (from cgMLST analysis)
883 by the Neighbor-Joining method, the Jukes-Cantor substitution model and 1000
884 bootstrapping. A total of 525 and 320 core genome polymorphic genes were obtained for *C.*
885 *coli* and *C. jejuni*, respectively. Bootstrapping values are indicated for each branch in blue
886 text. Isolate names were substituted by their Sequence Type (ST) whenever they are known
887 and kept for those isolates with unknown CC according to MLST analysis. Only ST-828
888 was found for *C. coli* isolates (indicated in the middle of the tree). (C) Summary of the
889 available information on the isolates of *C. coli*, “*C. jejuni* generalist lineages” and “*C. jejuni*
890 specialist lineages” (columns): aerotolerance phenotypes are shown in rows and biofilm
891 formation ability under aerobic conditions is shown through the color code within the pie
892 charts. The size of the piecharts is proportional to the number of isolates belonging to each
893 category.

894 Figure S1: Comparison of survival rate under stress conditions and biofilm formation ability
895 of 111 *Campylobacter* isolates based on the source of isolation. (A) Behavior under aerobic
896 stress; (B) Growth in microaerobic conditions; (C) Biofilm formation under aerobic and mi-
897 croaerobic conditions; Tolerance to (D) refrigeration, (E) freeze-thaw, (F) heat, (G) PAA,
898 and (H) NaCl stresses. Statistical significance was determined using the Wilcoxon test. (ns:
899 $p > 0.05$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$).

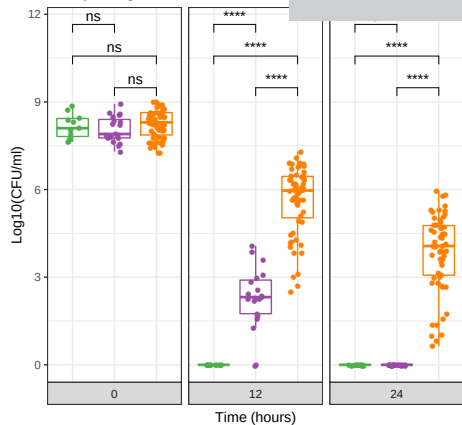
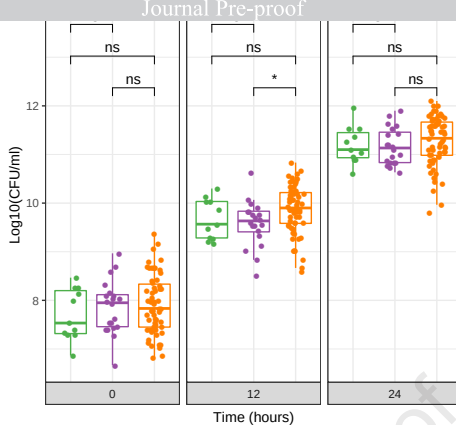
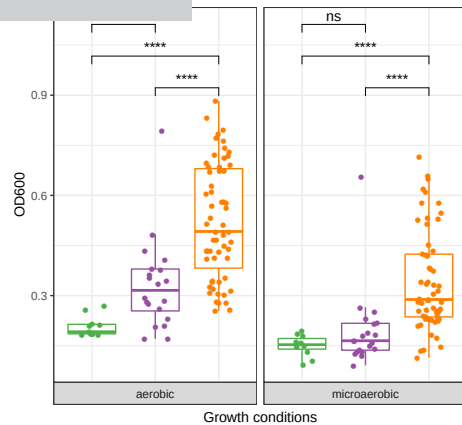
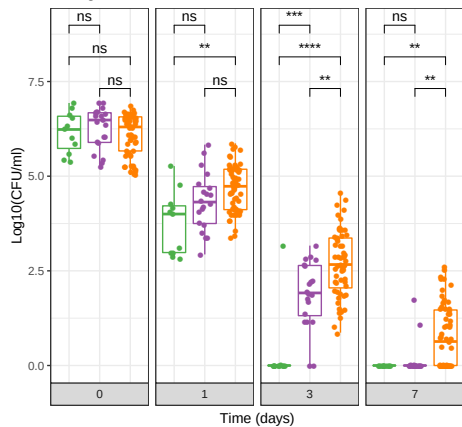
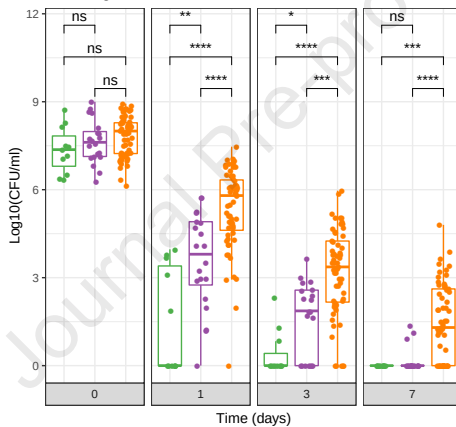
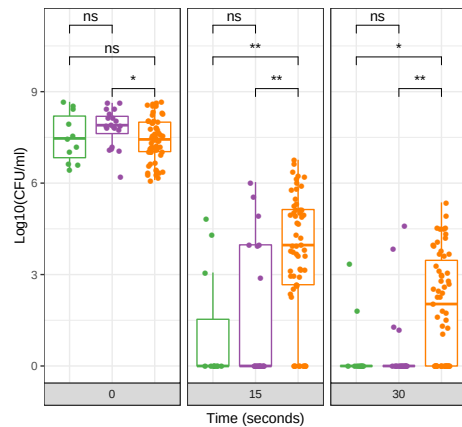
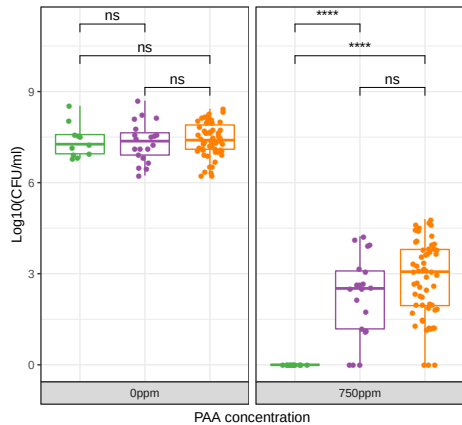
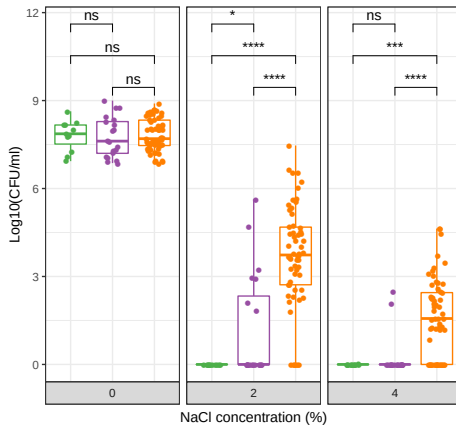
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901 Table S1: Bacterial isolates characteristics of 111 *Campylobacter* isolates and their survival
902 rates under six stress conditions (aerobic stress, refrigeration, peracetic acid, freeze-thaw,
903 heat, and NaCl) and biofilm formation potential under aerobic and microaerobic conditions.

904

905 Table S2: Whole genome sequence assembly quality features of 111 Egyptian *Campylobac-*
906 *ter* isolates.
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Journal Pre-proof

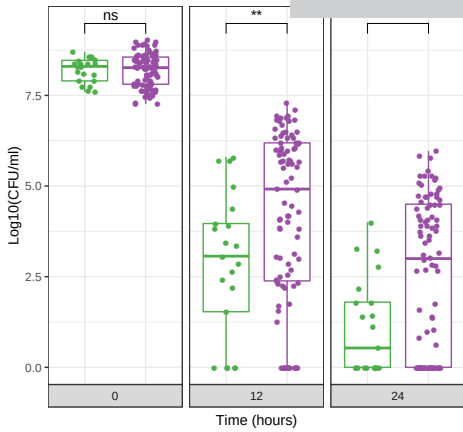
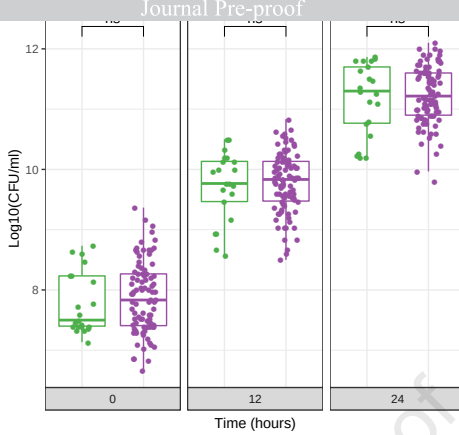
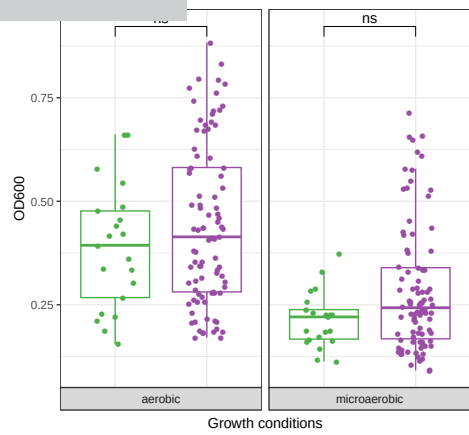
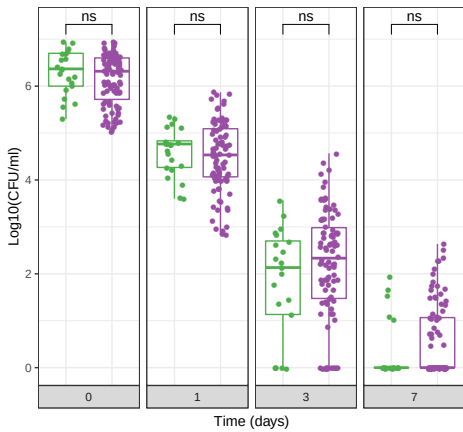
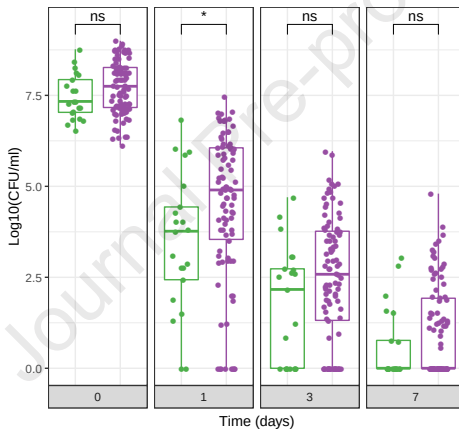
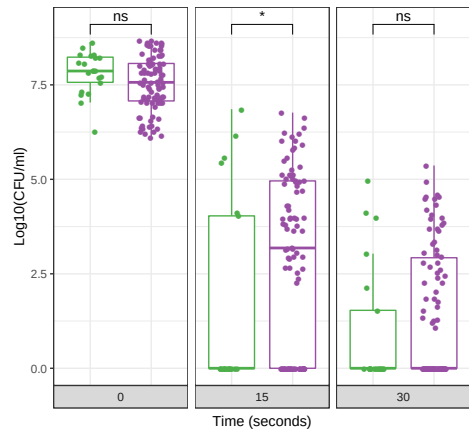
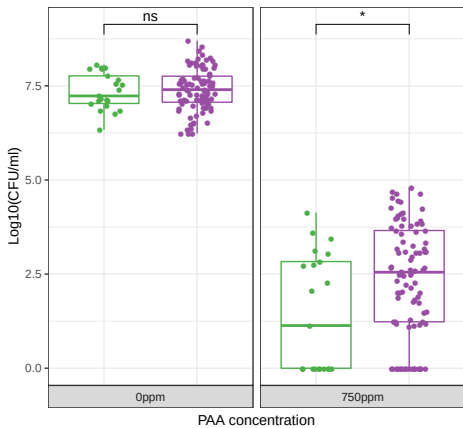
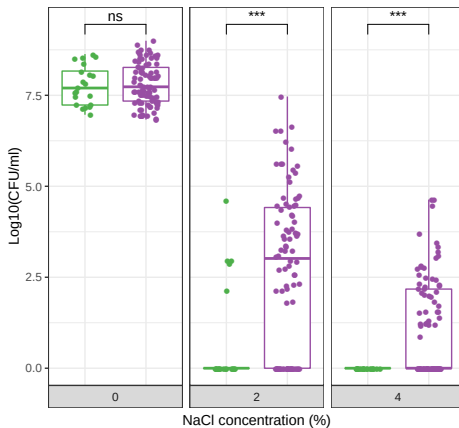
A Aerophilic growth**B** Microaerophilic growth**C** Biofilm formation**D** Refrigeration stress**E** Freezing stress**F** Heat stress**G** PAA stress**H** NaCl stress

Aerotolerance

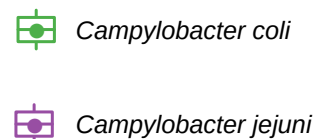
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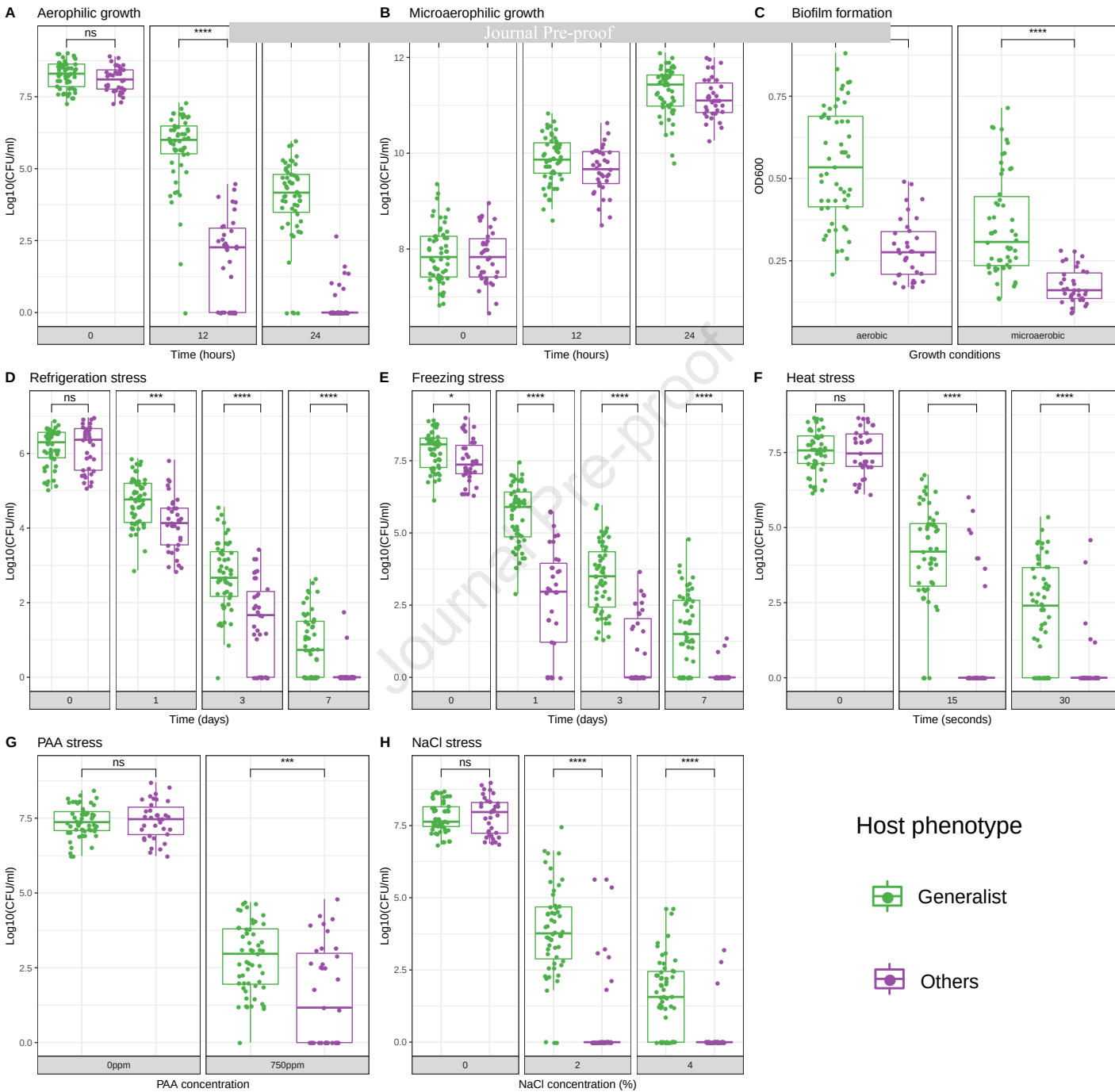
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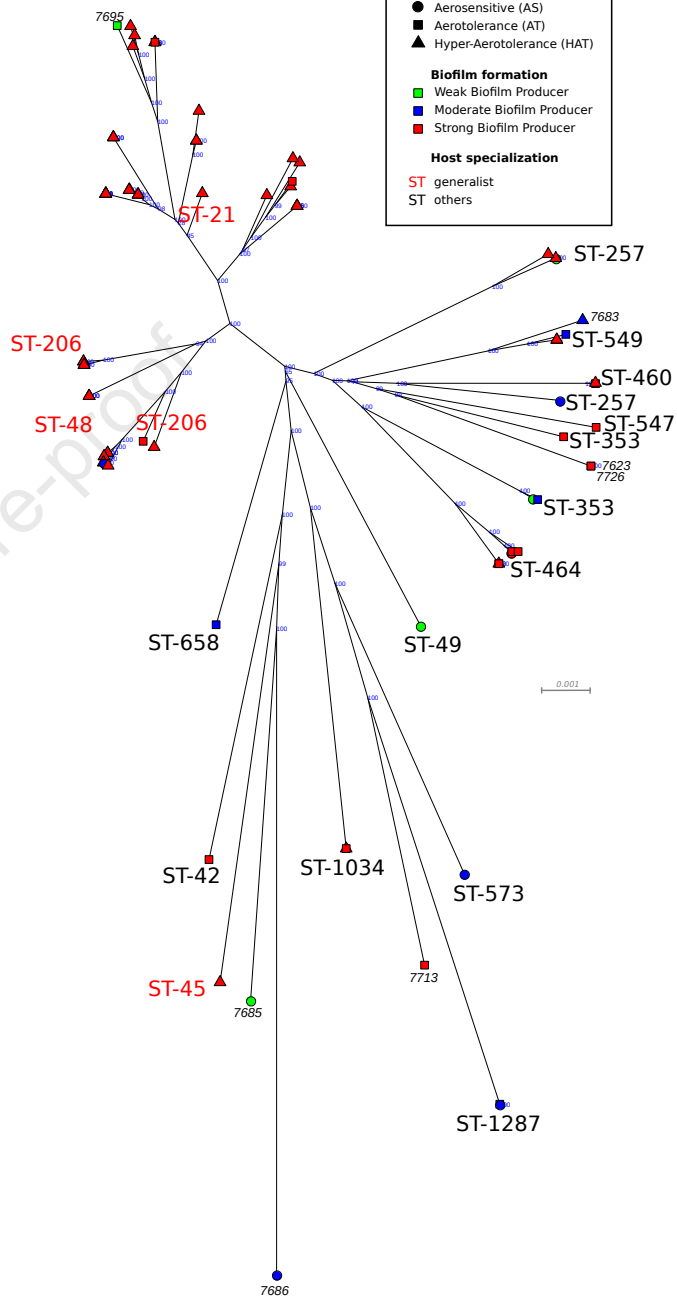
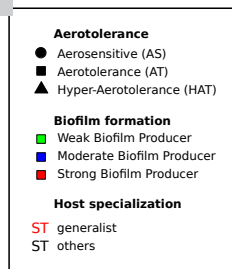
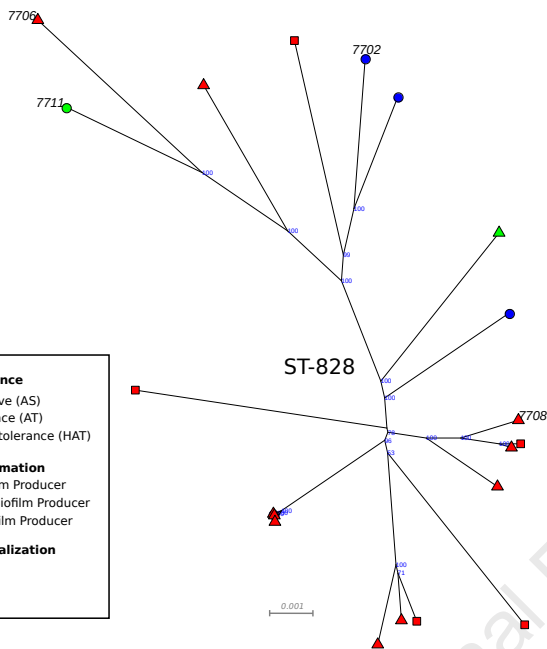
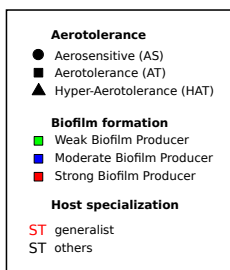
A Aerophilic growth**B** Microaerophilic growth**C** Biofilm formation**D** Refrigeration stress**E** Freezing stress**F** Heat stress**G** PAA stress**H** NaCl stress

Species

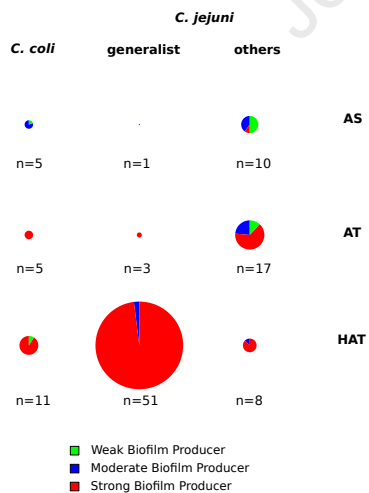




A



C



- Oxidative stress defense responses in *Campylobacter* are associated with enhanced biofilm formation capabilities
- Host generalist lineages are better equipped to withstand hostile environmental conditions favoring zoonotic transmission
- Multi-stress adapted *Campylobacter* isolates challenges efforts made to eliminate this foodborne pathogen from the food chain

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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