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Genotype and antibiotic resistance analysis of *Campylobacter* isolates from caeca and the carcasses of slaughtered broiler flocks

Simone E. Wirz, Gudrun Overesch, Peter Kuhnert, and Bożena M. Korczak*

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland

Running title: Genotyping of *Campylobacter* from broiler carcasses

*Corresponding author mailing address: Bożena Korczak, Institute of Veterinary Bacteriology, University of Bern, Laenggass-Str. 122, CH-3001 Bern, Switzerland.
Phone: 0041 (0)31 631 24 91
Fax: 0041 (0)31 631 26 34
E-mail: bozena.korczak@vbi.unibe.ch

ABSTRACT

To obtain genetic information about *Campylobacter jejuni* and *Campylobacter coli* from broilers and carcasses at slaughterhouses, we analyzed and compared 340 isolates that were collected in 2008 from the caecum right after slaughter or from the neck skin after processing. We performed *rpoB* sequence-based identification, multilocus sequence typing (MLST), and *flaB* sequence-based typing; we additionally analyzed mutations within the 23S rRNA and *gyrA* genes that confer resistance to macrolide and quinolone antibiotics, respectively. The *rpoB*-based identification resulted in a distribution of 72.0% *C. jejuni* and 28.0% *C. coli*. The MLST analysis revealed that there were 59 known sequence types (ST) and 6 newly defined STs. Most of the STs were grouped into 4 clonal complexes (CC) that are typical for poultry (CC21, CC45, CC257, CC828), and these represented 61.8% of all of the investigated isolates. The analysis of 95 isolates from the caecum and from the corresponding carcass neck skin covered 44 different STs, and 54.7% pairs had matching genotypes. The data indicate that cross-contamination from various sources during slaughter may occur, although the majority of *Campylobacter* contamination on carcasses appears to originate from the slaughtered flock itself. Mutations in the 23S rRNA gene were found in 3.1% of *C. coli*, although no mutations were found in *C. jejuni*. Mutations in the *gyrA* gene were observed in 18.9% of *C. jejuni* and 26.8% of *C. coli* isolates, which includes two *C. coli* strains that carried mutations conferring resistance to both classes of antibiotics. A relationship between specific genotypes and antibiotic resistance/susceptibility was observed.

Byline: multilocus sequence typing, genetic identification, macrolide resistance, quinolone resistance

INTRODUCTION

Campylobacteriosis is the leading food-borne bacterial gastroenteritis worldwide (12, 14). In Switzerland, the number of registered campylobacteriosis cases rapidly increased to more than 100 per 100,000 inhabitants in the past few years (15), and this trend has also been observed in the European Union (12). However, the real number of cases is likely higher, because not all cases are reported due to the self-limiting nature of the disease and potentially mild symptoms.

Campylobacter jejuni and *Campylobacter coli* are commonly associated with human infection, and they can be detected in up to 85% and 15% of cases, respectively (33). Despite the important role that *C. jejuni* and *C. coli* play as zoonotic pathogens worldwide, there is little information regarding the route(s) of transmission (17). Numerous case-control and modeling studies on the infection source of *C. jejuni* and *C. coli* have suggested that handling and consumption of contaminated poultry meat is associated with a risk of human campylobacteriosis (17, 45, 47, 49, 51). Initial meat contamination with *C. jejuni* or *C. coli* from the chicken intestine may occur during commonly used automated slaughter processing through several routes, such as the air, water, previously slaughtered flocks, or machinery (19, 36, 37).

Precise genotyping and continuous comparison of the strains obtained from, e.g., production site, flocks, slaughterhouse, retail meal, and infected humans, would help to trace the source of infection and may indicate possible intervention strategies for the contaminated site.

DNA sequence-based typing methods, such as multilocus sequence typing (MLST), are well-suited for this purpose (28), and MLST has become the method of choice for genotyping of *Campylobacter* (6, 8). Moreover, extension of the classical MLST technique for *C. jejuni* and *C. coli* with sequencing of the short variable region (SVR) within the flagellin-encoding gene *flaB* allows a more precise differentiation among strains that have the same MLST sequence

type (9, 29). An extended MLST workflow was recently developed that reduces the associated time and cost (24). In addition, the new approach allows genetic determination of antibiotic resistance to quinolones and macrolide. Resistance to these antibiotics is a worldwide issue of concern as an increasing number of *Campylobacter* isolates are resistant to these antibiotics. Strikingly, a number of strains are resistant to ciprofloxacin (quinolone) and to a lesser extent erythromycin (macrolide), which is problematic because these drugs are typically used to treat campylobacteriosis. Resistance to quinolone is mainly associated with a point mutation in the DNA gyrase gene (*gyrA*) at position C257T, and a transition in the 23S rRNA gene at position A2075G is commonly responsible for macrolide resistance (1). Simple sequence-based analysis of these common mutational positions can therefore provide information about the antibiotic susceptibility or resistance of a strain. Besides the prudent use of antibiotics, knowledge about the genetic composition of the infectious agent can be helpful to both treat the disease and prevent the spread of resistant strains.

In the current study, MLST, *flaB* typing, and sequence-based determination of quinolone and macrolide resistance were used to investigate the genetic background of *C. jejuni* and *C. coli* isolates collected from Swiss broiler in a spatiotemporal study in 2008. We adressed the following three aspects: i) the diversity of *Campylobacter* isolates that were recovered from pooled caecum samples and the carcass neck skin; ii) the possible impact of cross- and self-contamination during slaughter; and iii) the antibiotic resistance of *Campylobacter* strains from the broiler flocks and chicken carcasses. All of the data, including the strain information and trace files were entered into a commercial Web-based *Campylobacter* MLST database (SmartGene, Zug, Switzerland). This database allows users to retrieve and compare information for any analyzed strain for monitoring purposes (24).

MATERIALS AND METHODS

Sampling and template preparation. Broiler caecal and neck skin samples were collected during the EU baseline study on the prevalence of *Campylobacter* in broiler flocks (11), where Switzerland took part within the framework of the bilateral approach. Samples were obtained from 411 different flocks at 5 different suppliers in Switzerland (Table 1), which together cover approximately 80% of poultry meat production in the country. Each sample consisted of intact caeca from 10 birds and neck skin from one bird from the same slaughter batch. The samples were collected at the slaughterhouses once a week for at least 44 weeks during 2008. The caecal samples were taken at the time of evisceration by careful manual traction at the junction with the intestine. The neck skins were collected directly after chilling, but before further processing, such as freezing, cutting, or packaging (11). The samples were processed in the Center for Zoonosis, Bacterial Animal Diseases und Antibiotic Resistance in Bern (ZOBA) according to the EU recommendations (11). The neck skin batches were processed as previously described for qualitative detection of thermotolerant *Campylobacter* (43). For the caecal samples, the caecal content of 10 birds was aseptically removed and pooled. For direct cultivation, a loopful of the material was streaked onto a modified charcoal-cefoperazone-desoxycholate agar (mCCDA; Oxoid, Pratteln, Switzerland), as well as a campylosel (Oxoid) medium. The plates were incubated under microaerobic conditions at 41.5°C for 48 h. After initial cultivation of bacteria from the neck skin and the caeca batches, at least one presumptive *Campylobacter* spp. colony from each plate was subcultured on TSA medium (Oxoid) at 41.5°C for 24 to 48 h under microaerobic conditions. Isolates that belong to the genus *Campylobacter* were identified by microscopy, a positive oxidase test, and the absence of growth on TSA after 24-48 h under aerobic conditions at 41.5°C, as well as microaerobic incubation at 25°C. Phenotypic species identification was performed as previously reported by Schnider *et al.* (44). Hydrolysis of indoxyl acetate was used to differentiate the *Campylobacter* spp. from the *Helicobacter pullorum*, being negative for the latter. A cell lysate was prepared from each pure culture as described previously (24).

120 A total of 149 isolates from the caecum and 248 isolates from the neck skin were obtained
121 after cultivation and phenotypic identification. Isolate species identification through
122 phenotypic testing was verified by sequence analysis of the *rpoB* gene fragment. Only the
123 isolates confirmed as *C. jejuni* or *C. coli* were further investigated. The strain set allowed for a
124 comparison of 95 isolate pairs where there was cultivation of *Campylobacter* spp. from the
125 caeca and neck skin of the same slaughtered flock.
126
127 **Genotyping and determination of antibiotic resistance.** The *rpoB* gene fragment was
128 amplified with the broad-range primers CamrpoB-L, Pasrpob-L, and Rpob-R, and was
129 subsequently sequenced as the first stage of genetic characterization for species identification
130 (22, 23). The genotyping and antibiotic resistance analyses were performed as previously
131 described by Korczak *et al.* (24) with a few adaptations concerning the combination of target
132 genes in amplification groups. The 10 target genes were amplified in 3 multiplex PCRs: the
133 first multiplex PCR amplified the *glmM*, *aspA,* and the 23S rRNA gene fragments; the second
134 PCR amplified the *glnA*, *tkt*, *flaB,* and *gyrA* gene fragments; and the third PCR amplified the
135 *glyA*, *atpA,* and *gltA* gene fragments.
136
137 **Data analysis.** The data analysis was performed with the commercial Web-based application
138 for *Campylobacter* identification, typing and antibiotic resistance determination (IDNS
139 Campylobacter; SmartGene, Zug, Switzerland). In addition to the data entry and sequence
140 trace file editing, the sequence type (ST) and clonal complex (CC) were assigned with an
141 integrated automated link to the public PubMLST databank
142 (http://pubmlst.org/campylobacter). The CCs are defined as a group of independent isolates
143 that share at least four alleles (8). The genotypes of *flaB* were determined with a tool that is
144 provided by the PubMLST database (http://pubmlst.org/campylobacter). All of the new
145 genotypes were submitted directly to the curator of the PubMLST database. The BioNumerics

146 software, version 5.1 (Applied Maths NV, Sint-Martens-Latem, Belgium) was applied for
147 cluster analysis of concatenated sequences, whereas BLAST
148 (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was applied to compare the *rpoB* sequences and
149 identify species.
150
151 **Statistical analysis.** The binomial probability function of Statistix 9.0 for Windows
152 (Analytical Software, Tallahassee, FL) was used to calculate the probability values for
153 matching STs in the paired caecum and neck skin samples. The association of certain
154 genotypes with presence of quinolone resistance and their local distribution was tested by
155 Fisher's exact two-tailed test. A P value ≤ 0.05 was used to indicate statistically significant
156 results.
157 The Simpson's Index of Diversity was calculated as previously described (18).
158 The frequency distributions of STs that were obtained from the different suppliers were
159 compared by calculating the proportional similarity index (PSI), also known as the
160 Czekanowski index (42). The frequency distributions of the different sources were estimated
161 by calculating their similarity using the equation $PSI = 1 - 0.5 \sum_i |p_i - q_i|$, where p_i and q_i
162 indicate the proportion of strains that belong to ST i out of all of the strains that belong to the
163 source P and Q, respectively. A value of 1 indicates identical frequency distributions and a
164 value of 0 indicates no common types.
165 To investigate the population structure of the isolates from different suppliers, the correlated
166 allele frequency model of STRUCTURE 2.3 was used (13, 40). The population number (K)
167 was set as 5. The parameters were 100,000 burn-in iterations, followed by 100,000 sampling
168 iterations using the admixture model to determine the FST values.
169
170 **RESULTS**
171

172 **Genetic identification.** In most cases, phylogenetic *rpoB*-based analysis (23) confirmed the
173 results from the phenotypic identification of the isolates. There was a total of 245 *C. jejuni*, 95
174 *C. coli*, 54 *H. pullorum* and 3 *Arcobacter butzleri* recognized. The *rpoB* sequence similarities
175 between the *C. jejuni* and *C. coli*, *C. jejuni* and *H. pullorum*, and *C. jejuni* and *A. butzleri*
176 isolates varied from 83.17-98.97%, 71.79-74.07%, and 71.14-72.58%, respectively. The *C.*
177 *coli* isolates showed 71.79-75.73% *rpoB* sequence similarity to *H. pullorum* and 71.35-
178 73.83% sequence similarity to *A. butzleri*, whereas the *H. pullorum* and *A. butzleri* isolates
179 demonstrated 71.85-73.5% *rpoB* sequence similarity. Each new *rpoB* sequence was submitted
180 to the GenBank (Table 2). Twenty-one of the isolates (5.3%) had conflicting genotype and
181 phenotype data. Nine strains were phenotypically identified as *C. coli*, but were later
182 classified as *C. jejuni* because of their *rpoB* gene sequence identity (sequences identical to
183 *C. jejuni* Acc. No. CP000538, CP000814 or DQ174198). Five phenotypically identified *C.*
184 *jejuni* isolates were later classified as *C. coli* (sequences identical to *C. coli* Acc. No.
185 AF372098, HM486861 or HM486862). Two strains phenotypically identified as *H. pullorum*
186 were later identified as *C. coli* (sequence identical to Acc. No. AF372098) and *C. jejuni*
187 (sequence identical to Acc. No. CP000538) by *rpoB* gene analysis. The opposite was
188 observed with one *C. jejuni* and one *C. coli* isolate, where *rpoB* gene sequencing identified
189 the strains as *H. pullorum* (sequences identical to Acc. No. HM486886 and HM486887).
190 Finally, one *C. jejuni* and two *C. coli* isolates were genetically determined to be *A. butzleri*
191 (Table 2). Interestingly, *rpoB*-based identification of two indistinguishable paired isolates
192 (*i.e.*, the same strain) did not agree with the results from further genetic analysis. These
193 isolates were identified by *rpoB* sequencing as *C. jejuni* (*rpoB* sequence identical to Acc. No.
194 DQ174198), but all of the other target genes used for MLST and detection of antibiotic
195 resistance were specific for *C. coli*.
196 The prevalence of *C. jejuni* and *C. coli* isolated from the caecum and neck skin samples
197 differed slightly, and this difference was not significant. Within 114 caecal strains, 76 (66.7%)

198 were *C. jejuni* and 38 (33.3%) were *C. coli*; of the 226 neck skin samples, 167 (73.9%) were
199 *C. jejuni* and 59 (26.1%) were *C. coli*.
200
201 **Typing.** Of the 340 *Campylobacter* isolates that were analyzed, a total of 65 different STs
202 were identified (Table 1). Six of these were novel STs that comprised 18 (5.3%) isolates.
203 These STs have been submitted to the PubMLST database for appropriate number designation
204 and may be the result of new combinations of previously described alleles. However, for ST-
205 3991, a new *aspA* allele sequence was found and was designated 248. Twenty of the STs were
206 derived from *C. coli* isolates and 45 of the STs were observed within the *C. jejuni* isolates.
207 Eight STs were found only in isolates from the caecum and 26 STs were found only in the
208 isolates that were obtained from neck skin; all of the other STs were present in both sample
209 groups. Some of the isolates that belonged to the same ST could be further distinguished
210 when the entire amplicon sequence was compared. Differences within the regions that flank
211 the gene region that is defined as an allele by PubMLST were observed for ST-45, ST-122,
212 ST-267, and ST-1096.
213 The most frequent STs (number of isolates n≥ 14) within *C. jejuni* were ST-45 (n=34/14.0%),
214 ST-257 (n=27/11.1%), ST-50 (n=18/7.4%), ST-21 (n=17/7.0%), ST-48 (n=17/7.0%), and ST-
215 586 (n=14/5.8%). The new ST-3963 was observed within several of the *C. jejuni* isolates
216 (n=12/4.9%). The predominant STs within *C. coli* were ST-827 (n=32/33.0%) and ST-2142
217 (n=14/14.4%).
218 Fifty-five STs, which were present in 305 (89.7%) isolates, belonged to 20 previously defined
219 clonal complexes. The remaining 35 isolates were distributed among 10 STs, which could not
220 be assigned to any of the known lineages. The predominant clonal complexes were CC21
221 (18.5%), CC45 (18.9%), and CC257 (11.5%) for *C. jejuni*, and CC828 (93.8%) for *C. coli*.
222 Investigation of distribution of STs resulted in recognition of 7 different STs in 13 isolates
223 obtained from slaughterhouse A, 42 STs in 138 isolates obtained from slaughterhouse B, 40

224 STs in 125 isolates from slaughterhouse C, 10 STs in 26 isolates from slaughterhouse D, and
225 20 STs in 38 isolates obtained from slaughterhouse E (Table 1). There was a local association
226 between the ST-257 and the new ST-3963 that was statistically significant. ST-257 was the
227 most prominent ST in the sample set from slaughterhouse C (15.2%) compared to the others,
228 especially slaughterhouse B, which had a similar sample size but had only a 1.4%
229 representation of ST-257. ST-3963 was dominant in the samples that were obtained from
230 slaughterhouse D (34.6%).
231 The analysis of 95 paired samples identified 44 different STs. Whereas matching STs were
232 found in 52 (54.7%) of these samples, 43 (45.3%) of the paired samples did not have
233 matching STs (Fig. 1). Given the various STs that were observed, the probability to obtain
234 this high number of matches by chance is $p \simeq 0$ based on statistical analysis using binomial
235 probability.
236 Comparison of the *flaB* genotypes to the PubMLST database recognized 48 known and 11
237 new types (Fig. 2). Cluster analysis of the sequenced *flaB* fragments (446 base pairs) revealed
238 67 clusters (Fig. 2). This increase of resolution is due to the differences in flanking regions of
239 sequence used for allele assignation by the PubMLST database. In some cases, *flaB*
240 genotyping as well as sequence analysis allowed further separation of strains that belong to
241 the same ST, although different STs can be found with the same *flaB* sequence. In contrast to
242 the MLST data, there was no clear differentiation between the *flaB* types from *C. jejuni* and
243 *C. coli*, and in some cases, the two species had an identical *flaB* sequence. All 52 of the paired
244 samples that showed identical STs also had matching *flaB* sequences.
245 The Simpson's index of discrimination was calculated to be 0.958 for the MLST analysis
246 alone, 0.953 for the *flaB* genotype analysis, and 0.978 for the combination of both genotyping
247 methods. Analysis of the concatenated sequences of MLST gene fragments including the
248 flanking regions of alleles increased the discriminatory power slightly to 0.963 and increased

249 the combined MLST *flaB* value to 0.978, whereas the Simpson's index for the *flaB* sequence-
250 based analysis was 0.955.
251
252 **Genetic population structure.** The PSI was calculated to assess the similarity of ST
253 distribution between the five different slaughterhouses (Table 3). A relatively high value of
254 similarity was observed for ST distribution between suppliers B and C (PSI=0.579), which are
255 the two major producers in Switzerland. The PSI was also calculated for the caecum and neck
256 skin, and the resulting PSI of 0.697 indicates that there is a high overlap of ST distribution.
257 FST values based on the seven MLST alleles were determined for each of the 5
258 subpopulations with values for supplier A=0.008; supplier B=0.635; supplier C=0.511;
259 supplier D=0.720; and supplier E=0.671.
260
261 **Antibiotic resistance.** The point mutation C257T (corresponding to C150T in our fragment)
262 in the *gyrA* gene, which is associated with quinolone resistance, was observed in 46 *C. jejuni*
263 (18.9%) and 26 *C. coli* (26.8%) isolates. Twenty-three caecal isolates (13 *C. jejuni* and 10
264 *C. coli*) and 49 neck skin isolates (33 *C. jejuni* and 16 *C. coli*) carried this mutation. The
265 transition A2075G (corresponding to A227G in our fragment) in the 23S rRNA gene, which
266 contributes to macrolide resistance, was observed in 3 *C. coli* (3.1%) isolates. Two out of
267 these three *C. coli* strains carried point mutations in both genes, whereas none of the *C. jejuni*
268 isolates showed macrolide resistance.
269 Several specific genotypes showed significant association with quinolone resistance. Whereas
270 none of the CC45 ($P<0.01$), CC22 ($P<0.01$), CC257 ($P<0.01$), CC607 ($P<0.05$), or ST-586
271 ($P<0.05$) strains had a mutation in the *gyrA* gene, all isolates of the ST-464 ($P<0.01$), ST-829
272 ($P<0.01$), and ST-878 ($P<0.01$) strains had the specific mutation that confers quinolone
273 resistance.
274

DISCUSSION

In this study, we analyzed the genetic diversity and antibiotic resistance of *Campylobacter* in pooled caecum and neck skin samples from slaughter broilers and possible routes of carcass contamination at various slaughterhouses.

Proper phenotypic identification of *Campylobacter* isolates, especially differentiation between *C. jejuni* and *C. coli* based on the hippurate test might be difficult and could result in false isolate identification (35). Additionally, phenotypic discrimination between *Campylobacter* spp. and other phenotypically similar species that are present in the isolate sampling environment, such as *H. pullorum* and *A. butzleri,* can be difficult or even impossible (38). *rpoB* gene analysis allowed for individual species identification within the isolated strains; the data we obtained with this analysis corroborated with the results from phenotypic characterization (94.5%). Any minor disagreements between the genotype and phenotype data reflect the previously mentioned difficulties with interpretation of the phenotypic tests. The *rpoB*-based identification of *Campylobacter* could finally be confirmed by the species-specific STs determined by the MLST as well as by *gyrA* sequencing results that also allows phylogenetic separation of the two species (24). The only exception was one case of paired isolates (*i.e.*, the same strain) that possessed an *rpoB* gene sequence that matched *C. jejuni*, but had an ST and *gyrA* sequence that was specific for *C. coli*. This could have been caused by genetic recombination, which is known to occur between these highly related species (3, 46, 50). Thus, even though the *rpoB* gene sequence can be generally used to discriminate between the two species, false speciation can result from genetic recombination.

Roughly one-third of the isolates are *C. coli*, which corresponds to previously reported values from isolates in Swiss poultry (24, 53). Thus, both *C. jejuni* and *C. coli* are present in poultry samples and may cause campylobacteriosis in humans. Nevertheless, although both of these species are prevalent in poultry samples, less than 15% of *C. coli* is typically associated with

301 human disease (33). *C. coli* may therefore have a decreased ability to survive on broiler
302 carcasses, there could be a greater number of *C. jejuni* virulent strains, or other sources may
303 contribute to *C. jejuni* infection.
304 Within the sample set of 340 isolates, 65 known STs were assigned by MLST. Most of these
305 STs could be assigned to four main CCs (CC21, CC45, CC257, CC828). The CC21 and CC45
306 are also the most frequently reported *C. jejuni* genotypes in human disease and can be
307 detected in up to 21.8% and 44.6% of investigated cases, respectively (4, 21, 41). Strains
308 assigned to these lineages are common worldwide in a number of hosts, including poultry,
309 cattle, sheep, wild birds, and the natural environment (7, 21, 44). Therefore, identifying a
310 direct link between a specific carrier and campylobacteriosis may be difficult. In contrast,
311 CC257 can be identified in up to 24.2% of human cases and is mainly found in poultry
312 samples. Thus, human disease caused by *C. jejuni* assigned to this group probably occurs after
313 handling or consumption of contaminated poultry meat (4, 16, 24, 30, 44, 49).
314 The CC61 is identified frequently for cattle and sheep, and the feces of diseased humans (41,
315 45). Surprisingly, we identified the CC61 in one paired sample and two single neck skin
316 samples. Swiss agriculture is based on small mixed animal farms, thus strains can be easily
317 transmitted between different hosts (7). Because *C. coli* is most prevalent in swine, pork
318 products are considered to be the leading source of human infection (39). However, studies of
319 *C. coli* genetics have shown that only a small number of the genotypes found in swine can be
320 detected in samples from infected humans and other hosts (25, 47). In contrast, a considerable
321 genetic overlap between *C. coli* isolates from poultry and human was previously observed,
322 suggesting that infection was most likely due to the consumption of contaminated poultry
323 meat. Interestingly, the ST-827 which was the most prevalent genotype of the *C. coli* isolates
324 (33.0%) that were analyzed in this study, was also predominant (28.1%) in a set of *C. coli*
325 strains that were isolated from humans (48). Because this *C. coli* genotype is rare in isolates

326 from hosts other than chickens and humans, the ST-827 may have come from chicken meat
327 (6, 26, 31).
328 In contrast to recent study in New Zealand, population structure analyses of our set of strains
329 gave only little indications that specific populations can be discerned for a specific supplier
330 (34). The two major Swiss broiler suppliers analyzed (B and D) had a similar ST distribution
331 (PSI=0.579). An association between a particular sample supplier and several genotypes was
332 observed for two of the STs. ST-257 was most frequent genotype in the samples that were
333 collected from slaughterhouse C, and the novel genotype ST-3963 was found in 34.6% of
334 isolates from slaughterhouse D. A high FST value of 0.720 for the latter suggests that these
335 genotypes may represent a local clone. Further analyses of *Campylobacter* bacteria that are
336 present in delivery areas of both slaughterhouses would be interesting to test for the presence
337 of a local clone and attempt to track the source of campylobacteriosis.
338 MLST analysis of Swiss *Campylobacter* isolates has only recently been utilized.
339 Accumulation of more data about the strain genotypes from various sources will give a more
340 comprehensive picture about the distribution, population structure, and source of *C. jejuni* and
341 *C. coli* contamination in Switzerland.
342 We compared 95 sample pairs of cultivated *Campylobacter* isolates that were obtain from the
343 caecum and neck skin of the same flock. More than half of these pairs had matching STs.
344 Statistical analysis indicates that this is a highly significant association, and that the majority
345 of samples obtained from the neck skin originated from the slaughtered flock itself. Given the
346 fact that only single colonies were analyzed, and that both species and various genotypes can
347 be present in the flock, the true number of self-contaminated carcasses can be quite large (5,
348 52). The remaining non-matching isolates that were obtained from the neck skin could have
349 come from other contaminated sources, such as previously slaughtered batches, equipment,
350 working surfaces, or water (19, 36, 49). Additionally, cultivation of *Campylobacter* from the
351 caecum samples was unsuccessful in some cases. Interestingly, certain STs were found only

352 in the caecum, whereas others were found only on the neck skin. Whether this indicates that
353 environmental factors during the slaughter process lead to carcass contamination remains to
354 be more thoroughly investigated.
355 The *flaB*-based genotyping confirmed the results that were obtained with the MLST analysis
356 for the paired samples; pairs that had identical STs also had identical *flaB* sequences.
357 Generally, the resolution of the *flaB* sequence-based genotyping was slightly higher than the
358 MLST. In contrast to the sequence data from the MLST targets, the *flaB* sequence does not
359 allow phylogenetic analysis of the strains or differentiation between *C. jejuni* and *C. coli*.
360 However, it represents a different typing method that is useful for short-term investigations
361 due to the known instability of this marker, whereby *flaB* is more stable than the
362 conventionally used *flaA* (29). Sequence analysis of the entire amplicons of the MLST target
363 genes and *flaB* increases the discriminatory power only slightly, although this technique may
364 be useful to further differentiate between certain strains.
365 An increase in the number of *C. jejuni* and *C. coli* strains that are resistant to frequently used
366 antibiotics (macrolide and especially the quinolones) has been reported worldwide (1, 32). In
367 this study, no *C. jejuni* isolates and only 3.1% of the *C. coli* isolates were macrolide resistant,
368 whereas 18.9% *C. jejuni* and 26.8% *C. coli* isolates were resistant to quinolone (10).
369 Macrolide and the quinolones are allowed for use in veterinary medicine in Switzerland. A
370 high number of quinolone-resistant isolates could be associated with common therapeutic
371 application of enrofloxacin in broiler flocks; enrofloxacin treatment has been shown to induce
372 *Campylobacter* resistance and should therefore be used prudently (2, 20). However, the
373 number of antibiotic resistant *C. jejuni* and *C. coli* strains in Switzerland is in the lower range
374 of the European average reported antimicrobial resistance cases in poultry (27).
375 Remarkably, there was an association between specific genotypes and resistance to quinolone.
376 Because all of the broiler strains that belong to the genotypes ST-464, ST-829, and ST-878
377 carried the same point mutation within the *gyrA* gene that is responsible for quinolone

resistance, these *Campylobacter* genotypes are either prone to mutations within this gene or these STs are clonal. On the other hand, strains that belong to the frequently found clonal complexes CC45, CC22, CC257, CC607, and ST-586 appeared to be less susceptible to mutations within the *gyrA* gene. Previous studies of Belgian and Swiss *Campylobacter* reported similar results with CC45, where only single strains from this clonal complex were resistant to quinolone (16, 24). Additionally, 30% of the *C. jejuni* CC21 were resistant to quinolone, which was also seen in a previous study (16); this further suggests that there is a correlation between specific genotypes and antibiotic resistance.

In conclusion, we found that *C. coli* represented approximately one-third of the isolates, whereas *C. jejuni* represented the other two-thirds of the *Campylobacter* isolates that were obtained from Swiss slaughter broilers. The isolates showed high genetic variability in MLST and *flaB* genotyping with most common STs and CCs also described for poultry in other countries. A few genotypes showed local supplier association. Comparison of the paired samples indicates that there was mainly "self-contamination" of slaughtered broilers, although cross-contamination of carcasses cannot be excluded. Intervention at the production level could therefore have a most promising effect on the presence of *Campylobacter* in poultry products. Antibiotic resistance toward macrolide and quinolone in *C. jejuni* and *C. coli* seems less pronounced in Switzerland compared to other European countries. Nevertheless, the presence of antibiotic resistant strains indicates that such antibiotics should be used carefully, as overuse could lead to selection and spread of resistant bacteria.

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404 **REFERENCES**

405

406 1. **Alfredson, D. A. and V. Korolik**. 2007. Antibiotic resistance and resistance
407 mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol. Lett.
408 **277**:123-132.
409 2. **Anonymous**. 2009. Schweizer Zoonosenbericht 2008. Bundesamt für Veterinärwesen
410 BVet.
411 3. **Caro-Quintero, A., G. P. Rodriguez-Castano, and K. T. Konstantinidis**. 2009.
412 Genomic insights into the convergence and pathogenicity factors of *Campylobacter*
413 *jejuni* and *Campylobacter coli* species. J. Bacteriol. **191**:5824-5831.
414 4. **Colles, F. M., K. Jones, R. M. Harding, and M. C. Maiden**. 2003. Genetic diversity
415 of *Campylobacter jejuni* isolates from farm animals and the farm environment. Appl.
416 Environ. Microbiol. **69**:7409-7413.
417 5. **Colles, F. M., N. D. McCarthy, S. K. Sheppard, R. Layton, and M. C. Maiden**.
418 2010. Comparison of *Campylobacter* populations isolated from a free-range broiler
419 flock before and after slaughter. Int. J. Food Microbiol. **137**:259-264.
420 6. **Dingle, K. E., F. M. Colles, D. Falush, and M. C. J. Maiden**. 2005. Sequence typing
421 and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*.
422 J. Clin. Microbiol. **43**:340-347.
423 7. **Dingle, K. E., F. M. Colles, R. Ure, J. A. Wagenaar, B. Duim, F. J. Bolton, A. J.**
424 **Fox, D. R. Wareing, and M. C. Maiden**. 2002. Molecular characterization of
425 *Campylobacter jejuni* clones: a basis for epidemiologic investigation. Emerg. Infect.
426 Dis. **8**:949-955.
427 8. **Dingle, K. E., F. M. Colles, D. R. Wareing, R. Ure, A. J. Fox, F. E. Bolton, H. J.**
428 **Bootsma, R. J. Willems, R. Urwin, and M. C. J. Maiden**. 2001. Multilocus sequence
429 typing system for *Campylobacter jejuni*. J. Clin. Microbiol. **39**:14-23.

9. **Dingle, K. E., N. D. McCarthy, A. J. Cody, T. E. Peto, and M. C. Maiden**. 2008. Extended sequence typing of *Campylobacter* spp., United Kingdom. Emerg. Infect. Dis. **14**:1620-1622.

10. **Engberg, J., F. M. Aarestrup, D. E. Taylor, P. Gerner-Smidt, and I. Nachamkin**. 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. **7**:24-34.

11. **European Commission**. 2007. 2007/516/EC: Commission Decision of 19 July 2007 concerning a financial contribution from the Community towards a survey on the prevalence and antimicrobial resistance of *Campylobacter* spp. in broiler flocks and on the prevalence of *Campylobacter* spp. and *Salmonella* spp. in broiler carcasses to be carried out in the Member States (notified under document number C(2007) 3440). Official Journal - European Union Legislation L **50 (190)**:25-37.

12. **European Food Safety Authority**. 2009. The Community Summary Report on Foodborne Outbreaks in the European Union in 2007. The EFSA Journal **271**.

13. **Falush, D., M. Stephens, and J. K. Pritchard**. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes **7**:574-578.

14. **Friedman, C. R., J. Neimann, H. C. Wegener, and R. V. Tauxe**. 2000. Epidemiology of *Campylobacter jejuni* infections in the United States and other Industrialized Nations, p. 121-138. *In* I. Nachamkin and M. J. Blaser (eds.), *Campylobacter*. ASM Press, Washington, D.C.

15. **FVO**. 2009. Swiss Zoonosis Report 2008. Federal veterinary office FVO, Bern, Switzerland.

16. **Habib, I., W. G. Miller, M. Uyttendaele, K. Houf, and L. De Zutter**. 2009. Clonal population structure and antimicrobial resistance of *Campylobacter jejuni* in chicken meat from Belgium. Appl. Environ. Microbiol. **75**:4264-4272.

17. **Humphrey, T., S. O'Brien, and M. Madsen**. 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int. J. Food Microbiol. **117**:237-257.

18. **Hunter, P. R. and M. A. Gaston**. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26**:2465-2466.

19. **Jacobs-Reitsma, W., U. Lyhs, and J. Wagenaar**. 2008. *Campylobacter* in the food supply, p. 627-644. *In* I. Nachamkin, C. M. Szymanski, and M. J. Blaser (eds.), *Campylobacter*. ASM Press, Washington, DC.

20. **Jacobs-Reitsma, W. F., C. A. Kan, and N. M. Bolder**. 1994. The induction of quinolone resistance in *Campylobacter* bacteria in broilers by quinolone treatment. Lett. Appl. Microbiol **19**:228-231.

21. **Kärenlampi, R., H. Rautelin, D. Schonberg-Norio, L. Paulin, and M. L. Hanninen**. 2007. Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using Multilocus Sequence Typing, including comparison with epidemiological data and isolates from poultry and cattle. Appl. Environ. Microbiol. **73**:148-155.

22. **Korczak, B., H. Christensen, S. Emler, J. Frey, and P. Kuhnert**. 2004. Phylogeny of the family *Pasteurellaceae* based on *rpoB* sequences. Int. J. Syst. Evol. Microbiol. **54**:1393-1399.

23. **Korczak, B. M., R. Stieber, S. Emler, A. P. Burnens, J. Frey, and P. Kuhnert**. 2006. Genetic relatedness within the genus *Campylobacter* inferred from *rpoB* sequences. Int. J. Syst. Evol. Microbiol. **56**:937-945.

24. **Korczak, B. M., M. Zurfluh, S. Emler, J. Kuhn-Oertli, and P. Kuhnert**. 2009. Multiplex strategy for MLST, *fla*-typing and genetic determination of antimicrobial resistance of Swiss *Campylobacter jejuni* and *Campylobacter coli* isolates. J. Clin. Microbiol. **47**:1996-2007.

25. **Lang, P., T. Lefebure, W. Wang, B. P. Pavinski, R. J. Meinersmann, K. Kaya, and M. J. Stanhope**. 2010. Expanded multilocus sequence typing and comparative genomic hybridization of *Campylobacter coli* isolates from multiple hosts. Appl. Environ. Microbiol. **76**:1913-1925.

26. **Litrup, E., M. Torpdahl, and E. M. Nielsen**. 2007. Multilocus sequence typing performed on *Campylobacter coli* isolates from humans, broilers, pigs and cattle originating in Denmark. J. Appl. Microbiol. **103**:210-218.

27. **Luangtongkum, T., B. Jeon, J. Han, P. Plummer, C. M. Logue, and Q. Zhang**. 2009. Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. Future Microbiol. **4**:189-200.

28. **Maiden, M. C., J. A. Bygraves, E. Feil, G. Morelli, J. E. Russell, R. Urwin, Q. Zhang, J. Zhou, K. Zurth, D. A. Caugant, I. M. Feavers, M. Achtman, and B. G. Spratt**. 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A **95**:3140-3145.

29. **Mellmann, A., J. Mosters, E. Bartelt, P. Roggentin, A. Ammon, A. W. Friedrich, H. Karch, and D. Harmsen**. 2004. Sequence-based typing of *flaB* is a more stable screening tool than typing of *flaA* for monitoring of *Campylobacter* populations. J. Clin. Microbiol. **42**:4840-4842.

30. **Mickan, L., R. Doyle, M. Valcanis, K. E. Dingle, L. Unicomb, and J. Lanser**. 2007. Multilocus sequence typing of *Campylobacter jejuni* isolates from New South Wales, Australia. J. Appl. Microbiol. **102**:144-152.

31. **Miller, W. G., M. D. Englen, S. Kathariou, I. V. Wesley, G. Wang, L. Pittenger-Alley, R. M. Siletz, W. Muraoka, P. J. Fedorka-Cray, and R. E. Mandrell**. 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. Microbiology **152**:245-255.

32. **Moore, J. E., M. D. Barton, I. S. Blair, D. Corcoran, J. S. Dooley, S. Fanning, I. Kempf, A. J. Lastovica, C. J. Lowery, M. Matsuda, D. A. McDowell, A. McMahon, B. C. Millar, J. R. Rao, P. J. Rooney, B. S. Seal, W. J. Snelling, and O. Tolba**. 2006. The epidemiology of antibiotic resistance in *Campylobacter*. Microbes Infect. **8**:1955-1966.

33. **Moore, J. E., D. Corcoran, J. S. Dooley, S. Fanning, B. Lucey, M. Matsuda, D. A. McDowell, F. Mégraud, B. C. Millar, R. O'Mahony, L. O'Riordan, M. O'Rourke, J. R. Rao, P. J. Rooney, A. Sails, and P. Whyte**. 2005. *Campylobacter*. Vet. Res. **36**:351-382.

34. **Mullner, P., J. M. Collins-Emerson, A. C. Midwinter, P. Carter, S. E. Spencer, L. P. van der, S. Hathaway, and N. P. French**. 2010. Molecular epidemiology of *Campylobacter jejuni* in a geographically isolated country with a uniquely structured poultry industry. Appl. Environ. Microbiol. **76**:2145-2154.

35. **Nakari, U. M., A. Puhakka, and A. Siitonen**. 2008. Correct identification and discrimination between *Campylobacter jejuni* and *C. coli* by a standardized hippurate test and species-specific polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **27**:513-518.

36. **Nauta, M. J., W. F. Jacobs-Reitsma, and A. H. Havelaar**. 2007. A risk assessment model for *Campylobacter* in broiler meat. Risk Anal. **27**:845-861.

37. **Normand, V., M. Boulianne, and S. Quessy**. 2008. Evidence of cross-contamination by *Campylobacter* spp. of broiler carcasses using genetic characterization of isolates. Can. J. Vet. Res. **72**:396-402.

38. **On, S. L. W.** 1996. Identification methods for *Campylobacters*, *Helicobacters*, and related organisms. Clin. Microbiol. Rev. **9**:405-422.

39. **Payot, S., S. Dridi, M. Laroche, M. Federighi, and C. Magras**. 2004. Prevalence and antimicrobial resistance of *Campylobacter coli* isolated from fattening pigs in France. Vet. Microbiol. **101**:91-99.

40. **Pritchard, J. K., M. Stephens, and P. Donnelly**. 2000. Inference of population structure using multilocus genotype data. Genetics **155**:945-959.

41. **Ragimbeau, C., F. Schneider, S. Losch, J. Even, and J. Mossong**. 2008. Multilocus sequence typing, pulsed-field gel electrophoresis, and fla short variable region typing of clonal complexes of *Campylobacter jejuni* strains of human, bovine, and poultry origins in Luxembourg. Appl. Environ. Microbiol. **74**:7715-7722.

42. **Rosef, O., G. Kapperud, S. Lauwers, and B. Gondrosen**. 1985. Serotyping of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from domestic and wild animals. Appl. Environ. Microbiol **49**:1507-1510.

43. **Schnider, A., G. Overesch, B. M. Korczak, and P. Kuhnert**. 2010. Comparison of real-time PCR assays for detection, quantification, and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in broiler neck skin samples. J. Food Prot.

44. **Sheppard, S. K., J. F. Dallas, M. MacRae, N. D. McCarthy, E. L. Sproston, F. J. Gormley, N. J. Strachan, I. D. Ogden, M. C. Maiden, and K. J. Forbes**. 2009. *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. Int. J. Food Microbiol. **134**:96-103.

45. **Sheppard, S. K., J. F. Dallas, N. J. Strachan, M. MacRae, N. D. McCarthy, D. J. Wilson, F. J. Gormley, D. Falush, I. D. Ogden, M. C. Maiden, and K. J. Forbes**. 2009. *Campylobacter* genotyping to determine the source of human infection. Clin. Infect. Dis. **48**:1072-1078.

46. **Sheppard, S. K., N. D. McCarthy, D. Falush, and M. C. Maiden**. 2008. Convergence of *Campylobacter* species: implications for bacterial evolution. Science **320**:237-239.

556 47. **Siemer, B. L., E. M. Nielsen, and S. L. W. On**. 2005. Identification and molecular
557 epidemiology of *Campylobacter coli* isolates from human gastroenteritis, food, and
558 animal sources by amplified fragment length polymorphism analysis and Penner
559 serotyping. Appl. Environ. Microbiol. **71**:1953-1958.
560 48. **Sopwith, W., A. Birtles, M. Matthews, A. Fox, S. Gee, S. James, J. Kempster, M.**
561 **Painter, V. Edwards-Jones, K. Osborn, M. Regan, Q. Syed, and E. Bolton**. 2010.
562 Investigation of food and environmental exposures relating to the epidemiology of
563 *Campylobacter coli* in humans in Northwest England. Appl. Environ. Microbiol.
564 **76**:129-135.
565 49. **Wilson, D. J., E. Gabriel, A. J. Leatherbarrow, J. Cheesbrough, S. Gee, E. Bolton,**
566 **A. Fox, P. Fearnhead, C. A. Hart, and P. J. Diggle**. 2008. Tracing the source of
567 campylobacteriosis. PLoS Genet. **4**:e1000203.
568 50. **Wilson, D. J., E. Gabriel, A. J. Leatherbarrow, J. Cheesbrough, S. Gee, E. Bolton,**
569 **A. Fox, C. A. Hart, P. J. Diggle, and P. Fearnhead**. 2008. Rapid evolution and the
570 importance of recombination to the gastro-enteric pathogen *Campylobacter jejuni*. Mol.
571 Biol. Evol. **26**:385-397.
572 51. **Wingstrand, A., J. Neimann, J. Engberg, E. M. Nielsen, P. Gerner-Smidt, H. C.**
573 **Wegener, and K. Molbak**. 2006. Fresh chicken as main risk factor for
574 campylobacteriosis, Denmark. Emerg. Infect. Dis. **12**:280-285.
575 52. **Wittwer, M., J. Keller, T. M. Wassenaar, R. Stephan, D. Howald, G. Regula, and**
576 **B. Bissig-Choisat**. 2005. Genetic diversity and antibiotic resistance patterns in a
577 *Campylobacter* population isolated from poultry farms in Switzerland. Appl. Environ.
578 Microbiol. **71**:2840-2847.
579 53. **Zweifel, C., K. D. Scheu, M. Keel, F. Renggli, and R. Stephan**. 2008. Occurrence and
580 genotypes of *Campylobacter* in broiler flocks, other farm animals, and the environment

during several rearing periods on selected poultry farms. Int. J. Food Microbiol. **125**:182-187.

584 **FIGURE LEGENDS**

585

586 Figure 1. Clustering of the paired sample strains using the entire MLST target sequences. The
587 unweighted-pair group method using average linkages tree (UPGMA) was applied in
588 BioNumerics. The sequence type (ST) (I), *flaB* types (II), and suppliers' designation are
589 indicated. Paired samples (IV) are labeled with corresponding numbers with the caecum (C)
590 and neck skin (S). The numbers in brackets indicate identical STs that are variants based on
591 differences that were observed within flanking regions of the sequence used for allele
592 definition by PubMLST.

593

594 Figure 2. Clustering of the strains based on the partial *flaB* gene sequences. The unweighted-
595 pair group method using average linkages tree (UPGMA) was applied in BioNumerics. The
596 *flaB* type (I), species (II), and sequence type (ST) are indicated (III). The numbers in brackets
597 indicate variants of identical *flaB* types within flanking regions of the sequence used for allele
598 definition by PubMLST.

599 **TABLES**

600

601 Table 1. A summary of the MLST data, supplier, and number of Swiss broiler isolates that
602 were analyzed.

Origin	Species	Clonal complex	ST	A	B	C	D	E	*Total*
Caecum	*C. jejuni*	21	19	0	0	0	0	1	*1*
			21	0	4	1	1	1	*7*
			50	0	1	4	0	1	*6*
			262	0	1	0	0	0	*1*
			883	0	1	0	0	0	*1*
			917	0	1	0	0	0	*1*
		22	22	1	0	2	0	0	*3*
		45	45	2	7	2	0	1	*12*
			137	0	2	1	0	0	*3*
			1964	0	0	1	0	0	*1*
		48	48	1	5	2	1	1	*10*
		61	61	0	1	0	0	0	*1*
		206	122	0	2	1	0	0	*3*
		257	257	0	1	4	1	1	*7*
		283	267	0	1	0	0	0	*1*
			383	0	1	0	0	0	*1*
		354	354	0	1	0	0	0	*1*
			1073	0	0	0	0	1	*1*
		443	51	0	1	0	0	0	*1*
		460	2952	0	2	0	0	0	*2*
		607	**3963**	0	0	0	2	0	*2*
		ND	464	0	2	0	0	3	*5*
		ND	586	0	2	1	0	1	*4*
		ND	2655	0	1	0	0	0	*1*
	Total			*4*	*37*	*19*	*5*	*11*	*76*
	C. coli	828	825	0	2	1	2	0	*5*
			827	1	4	6	0	0	*11*
			829	0	0	0	0	1	*1*
			854	0	1	3	0	1	*5*
			1096	0	2	1	0	0	*3*
			1545	0	1	0	0	0	*1*
			1556	0	0	0	1	0	*1*
			1563	0	1	0	0	0	*1*
			1614	0	0	0	0	1	*1*
			2142	0	2	2	0	1	*5*
			3336	0	0	1	0	0	*1*
		ND	1049	0	0	0	0	1	*1*
		ND	1680	0	0	1	0	0	*1*
		ND	**3989**	0	1	0	0	0	*1*
	Total			*1*	*14*	*15*	*3*	*5*	*38*

603 Table 1. continued

Origin	Species	Clonal complex	ST	A	B	C	D	E	*Total*
		21	19	0	1	0	0	0	*1*
			21	0	5	3	2	0	*10*
			50	0	4	7	0	1	*12*
			262	0	1	1	0	0	*2*
			883	0	0	1	0	0	*1*
			917	0	0	1	0	0	*1*
			3988	0	0	1	0	0	*1*
		22	22	1	3	6	0	0	*10*
		42	42	0	2	0	0	0	*2*
		45	11	0	1	0	0	0	*1*
			45	1	8	11	0	2	*22*
			137	0	2	0	0	0	*2*
			418	0	1	0	0	0	*1*
			782	0	0	1	0	0	*1*
			1964	0	0	1	0	0	*1*
			2197	0	0	1	0	0	*1*
			2219	0	0	1	0	0	*1*
		48	48	0	4	3	0	0	*7*
		52	52	0	0	1	0	1	*2*
		61	61	0	2	1	0	0	*3*
		177	1388	0	1	0	0	0	*1*
		206	122	0	3	0	0	0	*3*
			227	0	0	1	0	0	*1*
			572	0	1	0	0	0	*1*
		257	257	0	1	15	0	4	*20*
			824	0	0	0	0	1	*1*
		283	267	0	2	1	0	2	*5*
			383	0	1	0	0	0	*1*
		353	353	0	2	0	0	0	*2*
		354	878	1	3	4	0	0	*8*
			1073	0	2	1	0	2	*5*
		362	587	0	0	1	0	0	*1*
		443	51	0	1	1	0	0	*2*
		460	2952	0	1	0	0	0	*1*
		607	607	0	1	1	0	0	*2*
			3963	0	1	2	7	0	*10*
		677	677	0	0	1	0	0	*1*
		1034	1956	1	0	0	0	0	*1*
		ND	441	0	0	1	1	0	*2*
		ND	464	0	3	0	0	2	*5*
		ND	586	0	5	3	0	2	*10*
		ND	1962	0	0	1	0	0	*1*
		ND	**3964**	0	0	0	0	1	*1*
Neck skin	***C. jejuni***								
Total				*4*	*62*	*73*	*10*	*18*	*167*

604 Table 1. continued

Origin	Species	Clonal complex	ST	A	B	C	D	E	*Total*
Neck skin	***C. coli***	828	825	0	1	2	4	0	*7*
			827	3	10	7	0	1	*21*
			829	0	3	0	0	1	*4*
			854	0	1	2	1	0	*4*
			1016	0	2	0	0	0	*2*
			1096	0	2	0	0	1	*3*
			1413	0	0	1	0	0	*1*
			1614	0	0	1	0	1	*2*
			2142	0	5	4	0	0	*9*
			3023	0	0	0	1	0	*1*
			3990	0	0	1	0	0	*1*
			3991	0	1	0	0	0	*1*
		ND	1584	0	0	0	2	0	*2*
		ND	**3989**	0	1	0	0	0	*1*
	Total			*3*	*26*	*18*	*8*	*4*	*59*

605 ND: Not defined

606 A-E indicate the slaughterhouses

607 New STs are indicated in bold

608 Table 2. The accession numbers and number of isolates that represent identical *rpoB* gene

609 fragment sequences.

Species	Acc. No.	Number of identical isolates
C. jejuni	**HM486850**	55
	HM486850	1
	HM486852	1
	HM486853	5
	HM486854	11
	CP000538	18
	HM486855	14
	HM486856	1
	DQ174198	87
	CP000814	49
	HM486857	1
	HM486858	1
	HM486859	1
C. coli	**HM486860**	7
	HM486861	2
	HM486862	6
	AF372098	78
	DQ174193	2
H. pullorum	**HM486863**	2
	HM486864	1
	HM486865	5
	HM486866	5
	HM486867	2
	HM486868	1
	HM486869	9
	HM486870	2
	HM486871	1
	HM486872	2
	HM486873	1
	HM486874	1
	HM486875	2
	HM486876	1
	HM486877	1
	HM486878	2
	HM486879	4
	HM486880	1
	HM486881	1
	HM486882	1
	HM486883	2
	HM486884	1
	HM486885	1
	HM486886	1
	HM486887	2
	HM486888	2
A. butzleri	**HM486889**	1
	HM486890	1
	HM486891	1

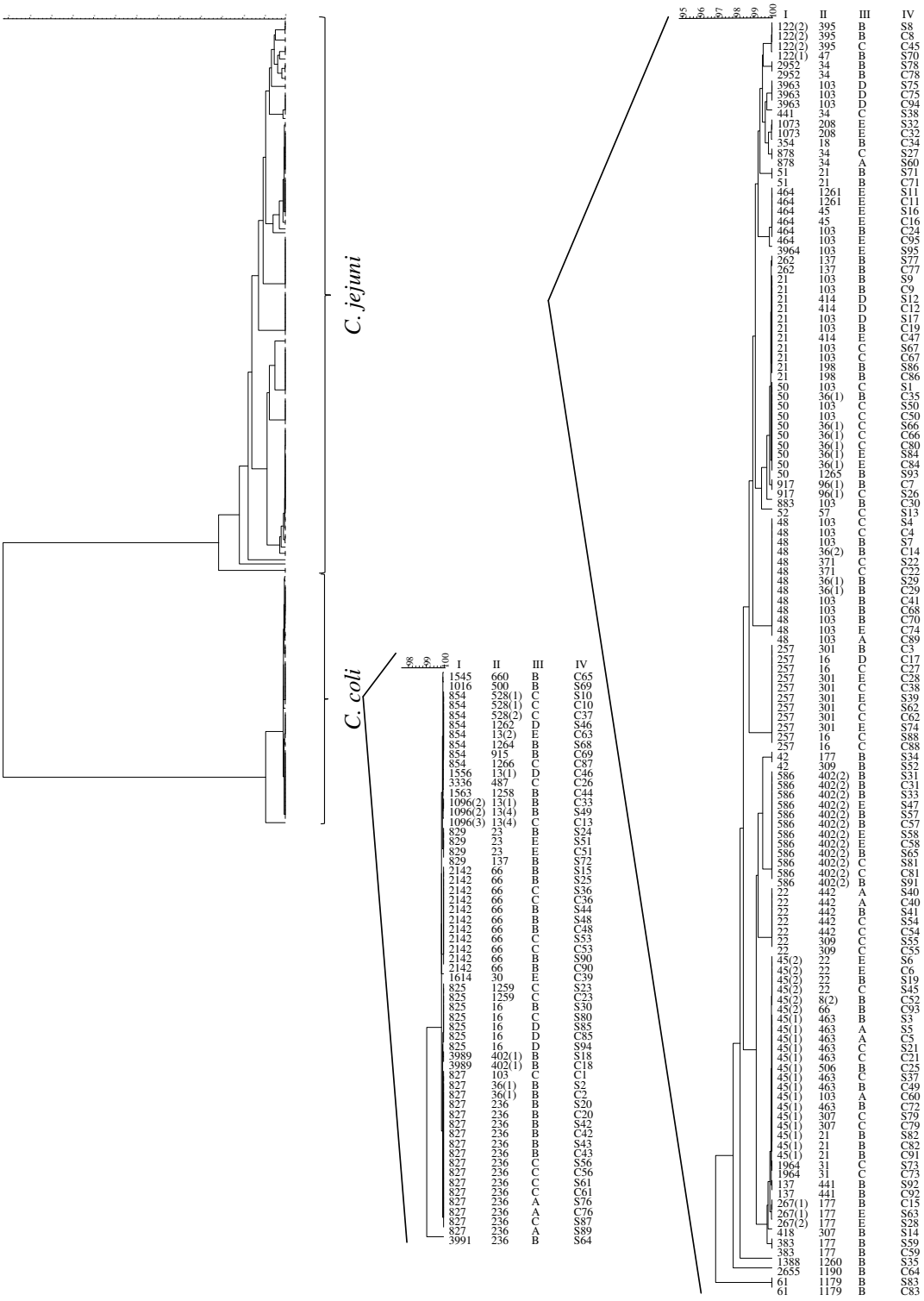
610 The accession numbers obtained in this study are indicated in bold

611 Table 3. The proportional similarity index of ST distribution between the five suppliers.

	Supplier A	Supplier B	Supplier C	Supplier D
Supplier B	0.317			
Supplier C	0.344	0.579		
Supplier D	0.038	0.161	0.195	
Supplier E	0.132	0.426	0.467	0.117

612

613



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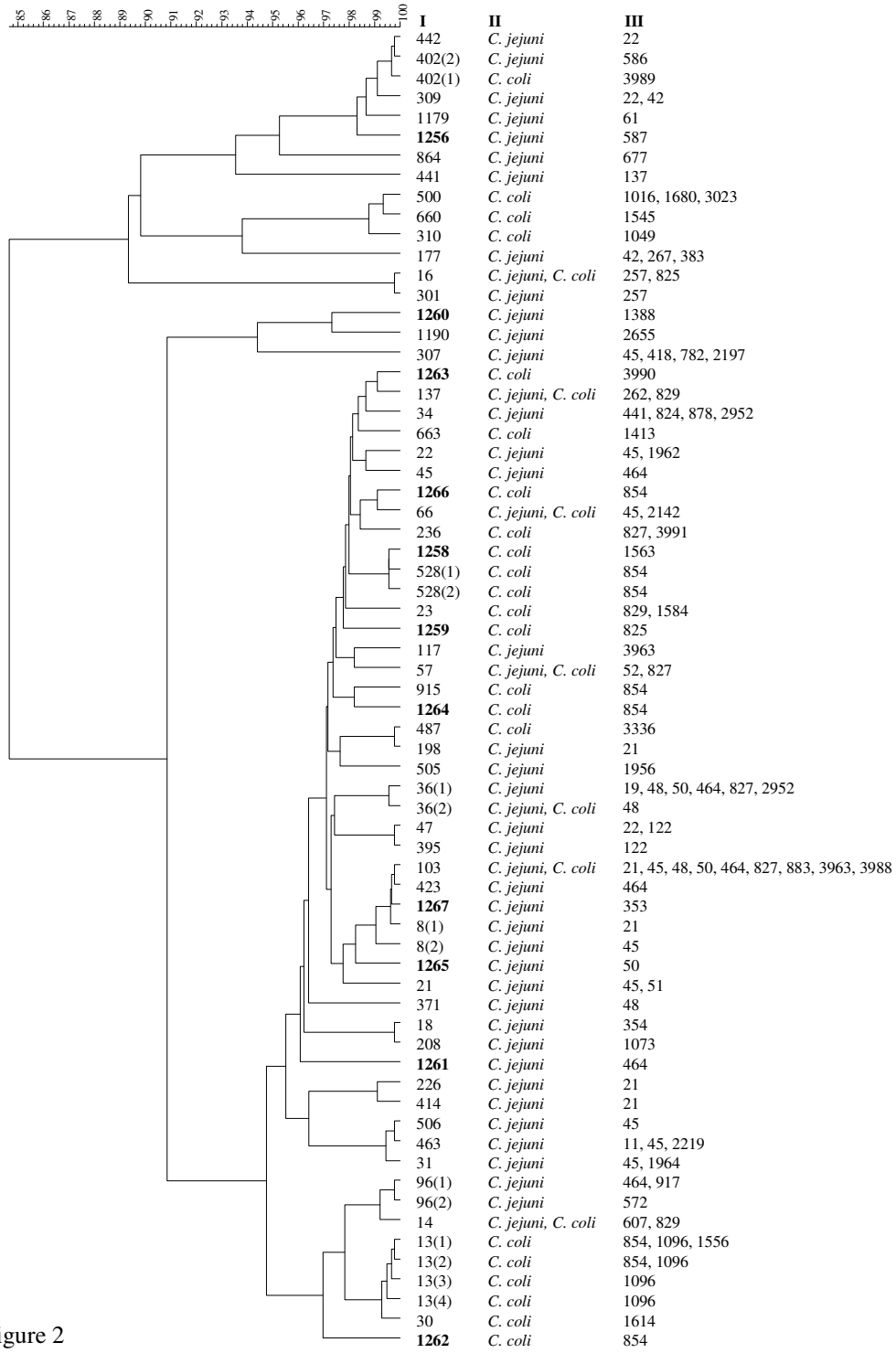

Wirz et al. Figure 2