Accepted Manuscript

Antimicrobial resistance in thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply chain in Argentina

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Please cite this article as: Zbrun M.V, Olivero C., Romero-Scharpen A., Rossler E., Soto L.P., Astesana D.M., Blajman J.E., Berisvil A., Signorini M.L. & Frizzo L.S., Antimicrobial resistance in thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply chain in Argentina, *Food Control* (2015), doi: 10.1016/j.foodcont.2015.03.045.

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22   Abstract

23   The objective of this study was to investigate the antimicrobial resistance in

24   thermotolerant *Campylobacter* spp. isolated from different stages of the poultry meat supply

25   chain in Argentina. Six poultry meat chains were studied from the reproductive farm to the

26   chicken at the retail. Chickens sampled along each food chain were from the same batch.

27   Samples collected were: a) cloacal samples from hens and chickens on the farm, b) chicken

28   carcasses from the slaughterhouse and retail market. Samples obtained were examined for

29   *Campylobacter* spp. Antimicrobial resistance was evaluated using the disk diffusion method.

30   Almost all isolates were resistant to nalidixic acid (91.2%) and ciprofloxacin (88.2%). A

31   large proportion of thermotolerant *Campylobacter* isolated from hens and broilers <1 wk

32   showed resistance to erythromycin in comparison with the rest of the stages of the poultry

33   meat supply chain (P= 0.031). *Campylobacter* isolated from broilers (both <1 wk and >5

34   wk) and carcasses at slaughterhouse and at retail showed a proportion of resistance to

35   ciprofloxacin and enrofloxacin higher than isolates from hens (P= 0.015 and P= 0.031,

36   respectively). One strain was resistant to all the antibiotics analyzed, and 46.1% of the

37   isolates were resistant to three or more drug classes. Almost 50% of the isolates were

38   resistant to all quinolones tested (ciprofloxacin, nalidixic acid, and enrofloxacin), and 13.2%

39   were resistant to all quinolones and erythromycin. *Campylobacter* strains isolated from

40   carcasses at retail showed higher resistance to all quinolones than strains isolated from hens

41   (P= 0.016). These results reflect an alarming situation with potential serious consequences

42   to the public health.

43   *Keywords*: antimicrobial resistance; *Campylobacter* spp.; poultry; zoonosis

44

1. Introduction

Diarrheal diseases are leading causes of childhood illness and death in developing countries (Zaidi et al., 2008). Thermotolerant *Campylobacter*, especially *Campylobacter jejuni*, is recognized as responsible for food-borne gastro-enteritis and diarrheal diseases worldwide (Avrain et al., 2003). In Argentina thermotolerant *Campylobacter* was found as the most important gastrointestinal pathogen in humans (with an incidence rate of 22.4% and 13.6% for children under 3 years and adults, respectively) (Fuentes, 2010). These pathogens are frequently found in the intestinal tract of a wide variety of wild and domesticated animals, especially birds and animal origin foods, in particular poultry meat, are known to be the most important source of human campylobacteriosis (Signorini et al., 2013; EFSA 2010).

Although most cases of campylobacteriosis may be asymptomatic or may cause diarrheas and other serious sequels such as Guillain-Barré syndrome (Avrain et al., 2003). Antimicrobial treatment is only necessary for systemic infections (or in immune-compromised patients) and severe or long-lasting *Campylobacter* infections (Luber et al., 2003). Macrolides and fluoroquinolones (particularly erythromycin and ciprofloxacin) are the first- and second-choice antimicrobials for that purpose. Intravenous aminoglycosides are also recommended for the treatment of serious *Campylobacter* bacteremia (Kassa et al., 2007). Resistance to antimicrobials, particularly to macrolides and fluoroquinolones, has been reported to be increasing (Avrain et al., 2003; Luber et al., 2003). In Argentina, only few studies have evaluated the antimicrobial resistance in *Campylobacter* strains (Tamborini et al., 2012; Pantozzi et al., 2010) with a low number of strains analyzed. Additionally, no epidemiological studies in Argentina have assessed the prevalence of *Campylobacter*

68 resistant to antibiotics on the whole food chain from farm to fork. This information is

69 essential to establish a public health program to control the disease and it is considered as a

70 starting point for a future monitoring program.

71   In poultry meat supply chain there are a variety of environments which

72 thermotolerant *Campylobacter* have to adapt to survive. Biofilm formation is a strategy for

73 *Campylobacter* survival in suboptimal conditions such as temperature variation, aerobic

74 atmosphere, and nutrient starvation (Brown et al., 2014). Biofilm appears to be an important

75 reservoir of viable planktonic cells resistant to antimicrobials (Bae et al., 2014).

76   Regarding the methods to evaluate the antimicrobial resistance, disk diffusion

77 method for aminoglycosides, quinolones, erythromycin, and tetracycline has showed a high-

78 level correlation with agar dilution method. Disk diffusion method was considered as a

79 reliable, easy-to-use and inexpensive method for susceptibility testing of thermotolerant

80 *Campylobacter* spp., especially for screening purposes (Luangtongkum et al., 2007).

81   The objective of this study was to investigate the antimicrobial resistance in

82 thermotolerant *Campylobacter* isolated from different stages of the poultry meat supply

83 chain in Argentina. The criteria used to interpret susceptibility testing results were only for

84 epidemiological monitoring purposes.

85

86 2. Materials and methods

87 2.1. Collection of *Campylobacter* isolates

88   A total of 152 thermotolerant *Campylobacter* were isolated from six poultry meat

89 supply chains in Santa Fe region in Argentina (Zbrun et al., 2013). The stages sampled in

90 each poultry meat chain were: a) hens from breeder flocks, b) broilers in flocks (aged <1 wk

4

91 and > 5 wk), c) chickens at the slaughterhouse, and d) chicken meat at the retail market. The

92 chickens sampled along the meat supply chain were from the same batch (defined as a group

93 of chickens from the same flock, sent to the same slaughterhouse at the same time, and sold

94 together at the same retail market). A total of 555 samples were collected from: a) cloacal

95 samples from hens (n= 75) and chickens <1 wk (n= 180) and >5 wk (n= 180) on the farms,

96 b) chicken carcasses from the slaughterhouse (6 – 7 wk of age) (n= 60) and the retail market

97 (n= 60).

98   *Campylobacter* spp. were isolated using the selective media Bolton Broth and

99 Preston Agar (Bolton and Coates, 1983). All incubations were performed under

100 microaerophilic conditions (5% O_2, 10% CO_2 and 85% H_2). Preliminary identification of

101 thermotolerant *Campylobacter* spp. was based on colony morphology, microscopic

102 appearance (curved Gram-negative bacilli with typical motility), and the following

103 phenotypic characteristics: oxidase and catalase production, and hippurate hydrolysis

104 reaction (Lior, 1984). All presumptive *Campylobacter* isolates were identified to the species

105 level (*C. jejuni* and *C. coli*) by multiplex PCR, as proposed by Vandamme et al. (1997). The

106 negative isolates were tested by PCR for *C. lari* (Klena et al., 2004). The positive isolates

107 were subsequently purified on Columbia blood agar and stored in glycerol broth (15%

108 glycerol and 85% serum broth) at -80 ºC (Terzolo et al., 1987).

109 2.2. Antimicrobial susceptibility testing

110   The antimicrobial sensitivity of *Campylobacter* isolates was tested by the disk

111 diffusion assay as recommended by the Clinical and Laboratory Standards Institute in the

112 standard M100-S23 (CLSI, 2013). Strains were removed from the freezer and streaked onto

113 Columbia blood agar and then incubated for 48 h at 42ºC under microaerophilic conditions.

114 Several colonies were transferred to a tube with 5 ml of Mueller-Hinton broth to reach a

115 standard inoculum adjusted to 0.5 McFarland. Sterile cotton-tipped swabs were used to

116 transfer the inocula onto Mueller-Hinton agar supplemented with 5% sheep blood.

117 Antimicrobial disks were added after drying the plates for 5 min. The antimicrobials tested

118 were: ampicillin (10 µg) (Britania), erythromycin (15 µg) (Britania), tetracycline (30 µg)

119 (Britania), nalidixic acid (30 µg) (Britania), enrofloxacin (5 µg) (Oxoid), ciprofloxacin (5

120 µg) (Britania), and gentamicin (10 µg) (Britania). The plates were incubated for 24 h at 42

121 ºC under microaerophilic conditions. *C. jejuni* ATCC 33560 was used as a reference strain.

122 The zones of growth inhibition were evaluated according to the standards of the National

123 Committee for Clinical Laboratory Standards (NCCLS) (Nobile et al., 2013). If an isolate

124 was resistant to three or more drug classes, it was considered multi-drug resistant.

125 2.3. Statistical analysis

126 Chi-squared and Fisher´s exact two-tailed test were used to compare, for each

127 antibiotic, the distributions of resistant *Campylobacter* isolates according to the stages of the

128 poultry meat supply chain, using Infostat software (Universidad Nacional de Córdoba,

129 Argentina).

130

131 3. Results

132 Table 1 shows the frequency of isolation of thermotolerant *Campylobacter* species

133 according to the stage of the poultry meat chain. Results showed the following distribution:

134 69.1% (n= 105) was *C. jejuni*, 28.3% (n= 43) was *C. coli*, and 2.6% (n= 4) of positive

135 samples were not possible to identify to species level. *C. jejuni* represented at least 68.2% of

136 the *Campylobacter* species in the samples collected from broilers in the farm (at both ages)

137   and carcasses at the slaughterhouse and the retail market. In contrast, the proportions of *C.*

138   *coli* and *C. jejuni* in the samples collected from hens were similar (47.2%).

139   The lowest resistance was observed for gentamicin (7.9%) and erythromycin

140   (28.3%), while almost all isolates were resistant to nalidixic acid (91.2%) and ciprofloxacin

141   (88.2%). Table 2 shows the frequency of *C. jejuni* and *C. coli* isolates resistant to the

142   antimicrobials tested. *C. coli* showed higher resistance for tetracycline than *C. jejuni* (*P*=

143   0.039). For the other antibiotics, the proportions of resistance were similar for both species

144   (*P*> 0.05) (Table 2). Regarding the four isolates of *Campylobacter* spp., that could not be

145   identified to species level, the four were resistant to ampicillin, two to erythromycin, one to

146   gentamicin, three to ciprofloxacin, three to nalidixic acid, three to tetracycline, and two to

147   enrofloxacin.

148   The proportion of thermotolerant *Campylobacter* isolates resistant to ampicillin,

149   gentamicin, nalidixic acid, and tetracycline was similar for all the stages of the poultry meat

150   supply chain (*P*> 0.05). Almost 50% of the thermotolerant *Campylobacter* spp. isolated

151   from hens were resistant to erythromycin, whereas the proportion of resistant isolates from

152   broilers >5 wk and carcasses at the slaughterhouse and the retail was lower than 21.4% (*P*=

153   0.010, *P*= 0.019, and *P*= 0.048, respectively). On the other hand, the proportion of

154   thermotolerant *Campylobacter* spp. isolated from broilers >5 wk and carcasses at the

155   slaughterhouse and at retail resistant to ciprofloxacin was higher than that of isolates from

156   hens (*P*= 0.018, P= 0.013, and *P*= 0.009, respectively). Additionally, the proportion of

157   thermotolerant *Campylobacter* strains isolated from carcasses at slaughterhouse and at retail

158   resistant to enrofloxacin was higher than that of isolates from hens (*P*= 0.011 and *P*= 0.052,

159   respectively). *Campylobacter* strains isolated from broilers >5 wk and carcasses at

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160   slaughterhouse and at retail showed <u>non-significant</u> differences in the proportion of

161   resistance to the antibiotics tested <u>($P>$ 0.05)</u> (Table 2).

162   Only two isolates were sensitive to all the antibiotics tested and one was resistant to

163   all the antibiotics analyzed. Thirty-one different resistance patterns were identified, being

164   ampicillin, ciprofloxacin, nalidixic acid, and tetracycline (n= 17; 11.2%), ampicillin,

165   ciprofloxacin, nalidixic acid, and enrofloxacin (n= 15; 9.9%), and ampicillin, ciprofloxacin,

166   nalidixic acid, tetracycline, and enrofloxacin (n= 15; 9.9%), the most common combinations

167   (Table 3). About 46.1% of the isolates were resistant to three or more drug classes.

168   Multidrug-resistant isolates were associated with the poultry meat supply chain stage ($P=$

169   0.013) but not with the *Campylobacter* species ($P=$ 0.099).

170   Quinolones and macrolides are the most important antibiotics used for the treatment

171   of human campylobacteriosis. Considering all the isolates studied, 44.7% (n= 68) were

172   resistant to all quinolones tested (ciprofloxacin, nalidixic acid, and enrofloxacin), and 13.2%

173   (n=20) were resistant to all quinolones and erythromycin (Table 3). The thermotolerant

174   *Campylobacter* spp. isolated from carcasses at retail showed higher resistance to all

175   quinolones than those isolated from hens ($P=$ 0.016). The other poultry meat supply stages

176   had a frequency of isolates resistant to quinolones ($P=$ 0.187) similar to that found in hens.

177   Additionally, we found no association between the frequency of isolates resistant to

178   quinolones and quinolones + erythromycin and the *Campylobacter* species ($P=$ 0.326 and

179    $P=$ 0.368, respectively).

180

181   4. Discussion

182   This study is one of the few reports on antibiotic susceptibility of *Campylobacter*

183   spp. isolated from different stages of the poultry meat supply chain in Argentina.

184   *Campylobacter* strains showed high susceptibility to gentamicin. Messad et al. (2014) also

185   reported this behavior and proposed that it could be the consequence of the limited use of

186   this antibiotic in poultry farms.

187   The thermotolerant *Campylobacter* strains tested in this study showed high resistance

188   to quinolones, specifically ciprofloxacin, nalidixic acid and enrofloxacin. Similar results

189   have been previously observed in other countries (Messad et al., 2014; Nobile et al., 2013;

190   Luber et al., 2003). The poultry reservoir has a fundamental role in the emergence of

191   quinolone-resistant strains of *Campylobacter* (Giacomelli et al., 2014). The frequent use of

192   sub-therapeutic doses of these antibiotics, either as prophylaxis, therapeutic doses or growth

193   promoters in poultry farms, has been proposed as the main reason for this high resistance

194   (Avrain et al., 2003; Messad et al., 2014). The widespread use of antibiotics in poultry

195   selects for antibiotic-resistant mutants which are able to spread throughout the meat supply

196   chain (Desmonts et al., 2004). Australia's government banned the use of quinolones in food-

197   producing animals and currently shows a low frequency of *Campylobacter* strains resistant

198   to fluoroquinolones (Wieczorek et al., 2013a). Additionally, it has been reported that flocks

199   treated with ionophores (as coccidicidals) had a higher proportion of *Campylobacter* strains

200   resistant to multiple antibiotics such as ampicillin, nalidixic acid, and tetracycline (Avrain et

201   al., 2003). All this suggests that the control tools for thermotolerant *Campylobacter*

202   multiresistant to antibiotics cannot be addressed only to the restriction or prohibition of

203   antimicrobial drugs in animal feed. New production systems and new health tools should be

9

204 designed to address the public health problem of thermotolerant *Campylobacter* and

205 antimicrobial resistance.

206   Many studies regarding the prevalence of antimicrobial-resistant *Campylobacter*

207 have been performed in different countries. Each of these studies have shown a particular

208 pattern of antimicrobial resistance, which may be explained by the variations in the use of

209 antibiotic, origin of the samples, isolation techniques, and antimicrobial susceptibility tests

210 (Nobile et al., 2013). It is thus difficult to compare the prevalences among countries. In that

211 sense, a standard guideline should be developed to establish a global surveillance and thus

212 allow monitoring this food-borne pathogen both currently and after the implementation of

213 effective control measures to avoid resistance dissemination through the food chain.

214   The most remarkable result of our study was the high frequency of *Campylobacter*

215 strains with multi-drug resistance, especially to the antibiotics considered as first-choice for

216 serious *Campylobacter* infections (ciprofloxacin and erythromycin). The isolation of a high

217 proportion of *Campylobacter* strains resistant to all quinolones and resistant to quinolones +

218 erythromycin provides evidence of the key role of raw poultry meat in the exposure risk to

219 antibiotic-resistant *Campylobacter* strains in humans. This situation demands the adoption of

220 appropriate risk management measures to control the antibiotic use in food animals (Nobile

221 et al., 2013) and in human therapeutic treatment.

222   Our results indicate that, with the exception of tetracycline, both *Campylobacter*

223 species had the same frequency of antibiotic resistance. Tetracyclines are considered the

224 second-choice treatment and high resistance to this antimicrobial may have a negative

225 impact on public health. Many studies (e.g. Nobile et al., 2013; Luber et al., 2003) have

226 reported the influence of *Campylobacter* species on the resistance to antibiotics. In general,

10

227 *C. coli* shows higher resistance than *C. jejuni* (Giacomelli et al., 2014). Apparently, *C. coli*

228 strains have an intrinsic ability to generate resistance against some antibiotics such as

229 erythromycin (Luber et al., 2003). Wieczorek et al. (2013b) found greater resistance to

230 antimicrobials for *C. coli* than for *C. jejuni* for tetracycline. On the other hand, Nobile et al.

231 (2013) found that, in general, *C. jejuni* shows higher resistance to ciprofloxacin, gentamicin,

232 and norfloxacin than *C. coli*.

233 In the present work, the *Campylobacter* strains isolated from the different stages of

234 the poultry meat supply chain presented the same frequency of antimicrobial resistance. The

235 colonization of broilers with thermotolerant *Campylobacter* seems to be unavoidable. The

236 high prevalence of these microorganisms was spreading throughout the poultry meat supply

237 chain. In this regard, it would be useful to conduct studies in order to investigate the

238 genotypic variation among strains of *Campylobacter* spp. isolated from different stages of

239 the poultry meat supply chain to evaluate whether the bacterial population was a stable

240 single genotype or had a mixed and changing profile according with the stage. However, the

241 strains isolated from hens showed higher resistance to erythromycin than the strains isolated

242 from the remaining stages of the poultry meat supply chain. On the other hand, the

243 frequency of resistance to quinolones (ciprofloxacin and enrofloxacin) was higher in the

244 strains isolated from poultry in the farm and from slaughterhouse and retail carcasses than in

245 the strains isolated from hens. Four possible explanations might be proposed to clarify this

246 finding: a) Both the use of antimicrobials (frequency, dose and type of antimicrobial used)

247 and other animal husbandry practices are completely different between hens and poultry

248 farms. This may impact on the selection pressure and the emergence of *Campylobacter*

249 strains resistant to specific antibiotics. This different selection pressure is responsible for

11

250   generating specific clusters of *Campylobacter* strains at these two stages (hens *vs.* the rest of

251   the stages) of the poultry meat supply chain. Argentina does not have a surveillance system

252   on the use of veterinary drugs that allows collecting data on sales of antimicrobial agents.

253   This surveillance system dedicated to monitoring drug on species level is needed to assess

254   the true levels of antimicrobial use and therefore analyze the possible consequences of their

255   use in the emergence of antimicrobial-resistant strains; b) hens have a higher life than

256   poultry and therefore greater potential exposure to antimicrobials applied for prophylactic or

257   therapeutic purposes; c) the biosecurity measures adopted by the parent flocks are much

258   stricter than in poultry farms. Broilers have more possibilities to be in contact with many

259   vectors (such as flies, rodents, and wild birds) which have been identified as reservoirs for

260   the transmission of antibiotic-resistant *Campylobacter* to poultry (Sippy et al., 2012). In

261   addition to the fact that the ecological conditions are different, the transmission of

262   *Campylobacter* spp. from hen farms to poultry farms through eggs is unlikely (Zbrun et al.,

263   2013); and d) poorly sanitized abiotic surfaces and juices released from avian meat, found in

264   slaughterhouse and retail environment, support the biofilms formation, which reintroduce

265   planktonic microorganisms resistant to antimicrobials. Additionally, *C. jejuni* has the ability

266   to form biofilms in the watering supplies and plumbing systems of animal husbandry

267   facilities and animal-processing plants (Bae et al., 2014; Pearson et al., 1993).

268   The horizontal genes transmission has been identified as a relevant mechanism in the

269   dissemination of antibiotic resistance in *Campylobacter* spp. Mobile genetic elements are

270   commonly involved in the horizontal gene transfer, allowing the bacteria to acquire foreign

271   DNA from the environment. *C. jejuni* has the ability to develops biofilms on different

272   abiotic surfaces as a mechanism to survive in suboptimal conditions, increasing the

273   resistance to antimicrobials. *Campylobacter* biofilms help to resist a variety of stressors

274   throughout poultry meat chain supply and appear to be an important reservoir of antibiotic-

275   resistant *C. jejuni* under normal growth conditions even without antimicrobial pressure (Bae

276   et al., 2014).

277   An increase in the presence of antibiotic-resistant strains has also been observed in

278   *Campylobacter* spp. isolated from human cases (WHO, 2014). This emphasizes that these

279   results reflect the behavior of *Campylobacter* spp. along the poultry meat supply chain

280   (Luber et al., 2003). Studies conducted in Argentina testing *Campylobacter* strains isolated

281   from humans have shown remarkable resistance to ciprofloxacin (>70%) and tetracycline

282   (30-40%), but high susceptibility to erythromycin (Tamborini et al., 2012; Fuentes, 2010).

283   Antimicrobials used massively in both human medicine and veterinary medicine can

284   generate a high level of resistance (Wieczorek et al., 2013a).

285   Although the agar dilution technique has been classified as the standard test for

286   *Campylobacter* (McDermott et al., 2004), here we used the disk diffusion test to investigate

287   antimicrobial resistance. Because of its convenience, flexibility, and low cost, disk diffusion

288   has been standardized (EUCAST, 2012) and used widely to test rapidly growing pathogens

289   such as *Enterobacteriaceae* and also modified to test some fastidious organisms (CLSI,

290   2013).

291

292   5. Conclusions

293   The finding of antibiotic-resistant (especially multi-drug resistant) *Campylobacter*

294   spp. reflects an alarming situation with potential serious consequences to the health of

295   poultry meat consumers. The frequency of antibiotic resistant *Campylobacter* was variable

13

296 in the different steps of the poultry meat chain supply. The use of antimicrobials in poultry

297 farms may generate a selection pressure and lead the emergency of *Campylobacter* strains

298 resistant against specific antibiotics. It is necessary to immediately implement husbandry

299 measures with the aim to guide the rational use of antibiotics in poultry production.

300 Argentina should establish a surveillance program to investigate the prevalence and

301 tendency in prevalence of antibiotic-resistance *Campylobacter* throughout the poultry meat

302 supply chain which may be the scientific basis to establish a risk management policy.

303

314

315 References

316 Avrain, L., Humbert, F., L´Hospitalier, R., Sanders, P., Vernozy-Rozand, C., Kempf, I.

317 (2003). Antimicrobial resistance in *Campylobacter* from broilers: association with

318 production type and antimicrobial use. *Veterinary Microbiology, 96*, 267-276.

319 Bae, J., Oh, E., Jeon, B. (2014). Enhanced transmission of antibiotic resistance in

320 *Campylobacter jejuni* biofilms by natural transformation. *Antimicrobial Agents and*

321 *Chemotherapy, 58*, 7573-7575.

322 Bolton, F.J., Coates, D. (1983). Development of a blood-free *Campylobacter* medium:

323 screening tests on basal media and supplements, and the ability of selected supplements to

324 facilitate aerotolerance. *Journal of Applied Bacteriology, 54*, 115-125.

325 Brown, H.L., Reuter, M., Salt, L.J., Cross, K.L., Betts, R.P., van Vliet, A.H. (2014). Chicken

326 juice enhaces surface attachment and biofilm formation of *Campylobacter jejuni. Applied*

327 *and Environmental Microbiology, 80*, 7053-7060.

328 Clinical and Laboratory Standards Institute (CLSI). (2013). Performance Standards for

329 Antimicrobial Susceptibility Testing Approved Standards M100-S23 (23th ed.) Wayne, Pa:

330 NCCLS.

331 EFSA. (2010). Analysis of the baseline survey on the prevalence of *Campylobacter* in

332 broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008,

333 Part A: *Campylobacter* and *Salmonella* prevalence estimates. *EFSA Journal, 8* (03), 1503.

334 EUCAST (2012). Clinical breakpoints, epidemiological cut-off (ECOFF) values and

335 EUCAST disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli*.

336 http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/Campylobact

337 er_ wide_consultation_August_2012.pdf (Accessed December 11, 2014).

338 Fuentes, L.S. (2010). Prevalencia y perfiles de sensibilidad en cepas de *Campylobacter* spp.

339 aisladas de diarreas en Córdoba, Argentina. *Gastroenterology, 12,* 2-5.

340 Giacomelli, M., Salata, C., Martini, M., Montesissa, C., Piccirillo, A. (2014). Antimicrobial

341 resistance of *Campylobacter jejuni* and *Campylobacter coli* from poultry in Italy.

342 *Microbiology Drug Resistance, 20,* 181-188.

343 Kassa, T., Gebre-Selassie, S., Asrat, D. (2007). Antimicrobial susceptibility patterns of

344 thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Veterinary*

345 *Microbiology, 119,* 82-87.

346 Klena, J.D., Parker, C.T., Knibb, K., Ibbitt, J.C., Devane, P.M.L., Horn, S.T., Miller, W.G.,

347 Konkel, M.E. (2004). Differentiation of *Campylobacter coli*, *Campylobacter jejuni*,

348 *Campylobacter lari*, and *Campylobacter upsaliensis* by a Multiplex PCR Developed from

349 the Nucleotide Sequence of the Lipid A Gene *lpxA*. *Journal of Clinical Microbiology, 42,*

350 5549–5557.

351 Lior, H. (1984). New, extended biotyping scheme for *Campylobacter jejuni*, *Campylobacter*

352 *coli* and *Campylobacter laridis*. *Journal of Clinical Microbiology, 20,* 636-640.

353 Luangtongkum, T., Morishita, T.Y., El-Tayeb, A.B., Ison, A.J., Zhang, O. (2007).

354 Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar

355 dilution and the agar disk diffusion methods. *Journal of Clinical Microbiology, 45,* 590-594.

356 Luber, P. Wagner, J., Hahn, H., Bartelt, E. (2003). Antimicrobial resistance in

357 *Campylobacter jejuni* and *Campylobacter coli* strains isolated in 1991 and 2001-2002 from

358 poultry and humans in Berlin, Germany. *Antimicrobial Agents Chemotherapy, 47,* 3825-

359 3830.

360 McDermott, P.F., Bodeis, S.M., Aarestrup, F.M., Brown, S., Traczewski, M., Fedorka-Cray,

361 P., Wallace, M., Critchley, I.A., Thornsberry, C., Graff, S., Flamm, R., Beyer, J., Shortridge,

362 D., Piddock, L.J., Ricci, V., Johnson, M.M.. Jones, R.N., Reller, B., Mirrett, S., Aldrobi, J.,

363 Rennie, R., Brosnikoff, C., Turnbull, L., Stein, G., Schooley, S., Hanson, R.A., Walker, R.D.

364 (2004). Development of a standardized susceptibility test for *Campylobacter* with quality-

365 control ranges for ciprofloxacin, doxycycline, erythromycin, gentamicin, and meropenem.

366 *Microbial Drug Resistance, 10,* 124-131.

367 Messad, S., Hamdi, T.M., Bouhamed, R., Ramdani-Bouguessa, N. (2014). Frequency of

368 contamination and antimicrobial resistance of thermotolerant *Campylobacter* isolated from

369 some broiler farms and slaughterhouses in the region of Algiers. *Food Control, 40,* 324-328.

370 Nobile, C.G.A., Costantino, R., Bianco, A., Pileggi, C., Pavia, M. (2013). Prevalence and

371 pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy.

372 *Food Control, 32,* 715-718.

373 Pantozzi, F.L., Moredo, F.A., Vigo, M.G.B., Giacoboni, G.I. (2010). Antimicrobial

374 resistance in indicator and zoonotic bacteria isolated from domestic animals in Argentina.

375 *Revista Argentina de Microbiología, 42,* 49-52.

376 Pearson, A.D., Greenwood, M., Healing, D., Rollins, M., Shahamat, M., Donaldson, J.,

377 Colwell, R.R. (1993). Colonization of Broiler Chickens by Waterborne *Campylobacter*

378 *jejuni. Applied and Environmental Microbiology, 59,* 987-996.

379 Signorini, M.L., Zbrun, M.V., Romero-Scharpen, A., Olivero, C., Bongiovanni, F., Soto,

380 L.P., Frizzo, L.S., Rosmini, M.R. (2013). Quantitative risk assessment of human

381 campylobacteriosis by consumption of salad cross-contaminated with thermophilic

382 *Campylobacter* spp. from broiler meat in Argentina. *Preventive Veterinary Medicine, 109,*

383 37-46.

384 Sippy, R., Sandoval-Green, C.M.J., Sahin, O., Plummer, P., Fairbanks, W.S., Zhang, Q.,

385 Blanchong, J.A. (2012). Occurrence and molecular analysis of *Campylobacter* in wildlife on

386 livestock farms. *Veterinary Microbiology, 157,* 369-375.

387 Tamborini, A.L., Casabona, L.M., Viñas, M.R., Asato, V., Hoffer, A., Farace, M.I., Lucero,

388 M.C., Corso, A., Pichel, M. (2012). *Campylobacter* spp.: prevalence and pheno-genotypic

389 characterization of isolates recovered from patients suffering from diarrhea and their pets in

390 La Pampa Province, Argentina. *Revista Argentina de Microbiología, 44,* 266-271.

391 Terzolo, H.R., Lawson, G.H.K., Angus, K.W., Snodgrass, D.R. (1987). Enteric

392 *Campylobacter* infection in gnotobiotic calves and lambs. *Research in Veterinary Science,*

393 *43,* 72-77.

394 Vandamme, P., van Doorn, L.-J., Rashid, S.T.A., Quint, W.G.V., Plas, J.v.d., Chan, V.L.,

395 On, S.L.W. (1997). *Campylobacter hyoilei* Alderton et al. 1995 and *Campylobacter coli*

396 Veron and Chatelain 1973 are subjective synonyms. Int. J. Syst. Bacteriol. 47:1055-1060.

397 WHO (World Health Organization). (2014). Antimicrobial resistance. Global report on

398 surveillance. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf.

399 Wieczorek, K., Dykes, G.A., Osek, J., Duffy, L.L. (2013a). Antimicrobial resistance and

400 genetic characterization of *Campylobacter* spp. from three countries. *Food Control, 34,* 84-

401 91.

402 Wieczorek, K., Kania, I., Osek, J. (2013b). Prevalence and antimicrobial resistance of

403 *Campylobacter* spp. Isolated from poultry carcasses in Poland. *Journal of Food Protection,*

404 *76,* 1451-1455.

405   Zaidi, M.B., Calva, J.J., Estrada-García, M.T., León, V., Vázquez, G., Figueroa, G., López,

406   E., Contreras, J., Abbott, J., Zhao, S., McDermott, P., Tollefson, L. (2008). Integrated food

407   chain surveillance system for *Salmonella* spp. in Mexico. *Emergency Infectious Diseases,*

408   *14,* 429-435.

409   Zbrun, M.V., Romero-Scharpen, A., Olivero, C., Rossler, E., Soto, L.P., Rosmini, M.R.,

410   Sequeira, G.J., Signorini, M.L., Frizzo, L.S. (2013). Occurrence of thermotolerant

411   *Campylobacter* spp. at different stages of the poultry meat supply chain in Argentina. *New*

412   *Zealand Veterinary Journal, 61,* 337-343.

413

414   TABLES

415   Table 1: Distribution of thermotolerant *Campylobacter* spp. according to the stage of the

416   poultry meat supply chain.

417   Table 2: Antibiotic resistance of thermotolerant *Campylobacter* spp. at different stages of

418   the poultry meat supply chain.

419   Table 3: Antimicrobial resistance patterns of thermotolerant *Campylobacter* isolates.

Table 1: Distribution of thermotolerant Campylobacter spp. according to stage of poultry meat supply.

Campylobacter species	Stage of poultry meat supply n (%)				
	Hens	Broilers (<1 wk)	Broilers (>5 wks)	Carcass at slaughterhouse	Carcass at retail
Campylobacter spp.	2 (5.6)	-	1 (2.5)	1 (2.3)	-
C. jejuni	17 (47.2)	9 (81.8)	32 (80.0)	32 (74.4)	15 (68.2)
C. coli	17 (47.2)	2 (18.2)	7 (17.5)	10 (23.3)	7 (31.8)
TOTAL	**36 (23.7)**	**11 (7.2)**	**40 (26.3)**	**43 (28.3)**	**22 (14.5)**

Table 2: Antibiotic resistance of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain.

Stage of poultry meat supply	*Campylobacter* spp.	Antibiotic resistance n (%)						
		AMP	ERY	GEN	CIP	NA	TET	ENR
Hens	*C. jejuni* (n=17)	10 (58.8)	9 (52.2)	4 (23.5)	9 (52.9)	12 (70.6)	8 (47.1)	7 (41.2)
	C. coli (n=17)	16 (94.1)	8 (47.1)	1 (5.9)	15 (88.2)	15 (88.2)	14 (82.4)	5 (29.4)
	TOTAL (n=34)	**26 (76.5)**	**17 (50.0)**	**5 (14.7)**	**24 (70.6)**	**27 (79.4)**	**22 (64.7)**	**12 (35.3)**
Broilers (<1 wk)	*C. jejuni* (n=9)	6 (66.7)	5 (55.6)	2 (22.2)	7 (77.8)	9 (100)	5 (55.1)	6 (66.7)
	C. coli (n=2)	2 (100)	0 (0.0)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50.0)
	TOTAL (n=11)	**8 (72.7)**	**5 (45.5)**	**4 (18.2)**	**9 (81.8)**	**11 (100)**	**7 (63.6)**	**7 (63.6)**
Broilers (>5 wks)	*C. jejuni* (n=32)	12 (37.5)	3 (9.4)	1 (3.1)	30 (93.8)	31 (96.9)	17 (53.1)	16 (50.0)
	C. coli (n=7)	4 (57.1)	3 (42.9)	1 (14.3)	7 (100)	7 (100)	6 (85.7)	3 (50.0)
	TOTAL (n=39)	**16 (41.0)**	**6 (15.4)**	**2 (5.1)**	**37 (94.9)**	**38 (97.4)**	**23 (59.0)**	**19 (50.0)**
Carcasses at slaughterhouse	*C. jejuni* (n=32)	20 (62.5)	8 (25.0)	2 (6.3)	30 (93.8)	30 (93.8)	15 (46.9)	21 (85.6)
	C. coli (n=10)	7 (70.0)	1 (10.0)	0 (0.0)	9 (90.0)	10 (100)	5 (50.0)	2 (20.0)
	TOTAL (n=42)	**27 (64.3)**	**9 (21.4)**	**2 (4.8)**	**39 (92.9)**	**40 (95.2)**	**20 (47.6)**	**23 (54.8)**

		AMP	ERY	GEN	CIP	NA	TET	ENR
Carcasses at retail	C. jejuni (n=15)	9 (60.0)	3 (20.0)	0 (0.0)	15 (100)	13 (86.7)	12 (80.0)	10 (66.7)
	C. coli (n=7)	3 (42.9)	1 (14.3)	0 (0.0)	7 (100)	7 (100)	5 (71.4)	5 (71.4)
	TOTAL (n=22)	**12 (54.5)**	**4 (18.2)**	**0 (0.0)**	**22 (100)**	**20 (90.2)**	**17 (77.3)**	**15 (88.2)**
All poultry meat supply	C. jejuni (n=105)	57 (54.3)	28 (26.7)	9 (8.6)	91 (86.7)	95 (90.5)	57 (54.3)	60 (57.1)
	C. coli (n=43)	32 (74.4)	13 (30.2)	2 (4.7)	40 (93.0)	41 (95.3)	32 (74.4)	16 (38.1)
	P=	0.059	0.739	0.571	0.307	0.411	0.039	0.096

References: AMP, ampicillin; ERY, erythromycin; GEN, gentamycin; CIP, ciprofloxacin; NA,nalidixic acid; TET, tetracycline; ENR, enrofloxacin.

Table 3: Antimicrobial resistance patterns of thermotolerant *Campylobacter* isolates.

Nº of antimicrobials	Resistance patterns	Classes of Antibiotic in pattern (n)	Strains (n)	Total strains n (%)
Two antibiotics	CIP, NA	1	10	
	AMP, NA	2	3	
	AMP, CIP	2	1	
	AMP, ERY	2	1	17 (11.2)
	ERY, TET	2	1	
	GEN, NA	2	1	
Three antibiotics	**CIP, NA, TET**	2	14	
	CIP, NA, ENR	1	8	
	AMP, CIP, NA	2	6	
	AMP, CIP, ENR	2	2	34 (22.4)
	ERY, CIP, NA	2	2	
	AMP, CIP, TET	3	1	
	GEN, NA, ENR	2	1	
Four antibiotics	**AMP, CIP, NA, TET**	3	17	
	AMP, CIP, NA, ENR	2	15	
	CIP, NA, TET, ENR	2	8	
	ERY, NA, TET, ENR	3	3	
	AMP, ERY, TET, CIP	4	2	50 (32.9)
	ERY, CIP, NA, TET	3	2	
	ERY, CIP, NA, ENR	2	2	
	AMP, ERY, CIP, ENR	3	1	
Five antibiotics	**AMP, CIP, NA, TET, ENR**	3	15	
	AMP, ERY, CIP, NA, TET	4	4	25 (16.4)
	ERY, CIP, NA, TET, ENR	3	3	

	AMP, ERY, CIP, NA, ENR	3	2	
	ERY, GEN, CIP, NA, TET	4	1	
Six antibiotics	AMP, ERY, CIP, NA, TET, ENR	4	12	
	AMP, ERY, GEN, NA, TET, ENR	5	5	20 (13.2)
	AMP, ERY, GEN, CIP, NA, TET	5	2	
	AMP, GEN, CIP, NA, TET, ENR	4	1	
Seven antibiotics	AMP, ERY, GEN, CIP, NA, TET, ENR	5	1	1 (0.7)

References: AMP, ampicillin (β-lactam class); ERY, erythromycin (macrolide class); GEN, gentamycin (aminoglycoside class); CIP, ciprofloxacin (quinolone class); NA, nalidixic acid (quinolone class); TET, tetracycline (tetracycline class); ENR, enrofloxacin (quinolone class).