AEM
Journals.ASM.org

Comparison of Genotypes and Antibiotic Resistances of *Campylobacter jejuni* and *Campylobacter coli* on Chicken Retail Meat and at Slaughter

Sonja Kittl,[a] Bożena M. Korczak,[a] Lilian Niederer,[a] Andreas Baumgartner,[b] Sabina Buettner,[c] Gudrun Overesch,[a] Peter Kuhnert[a]

Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland[a]; Federal Office of Public Health, Bern, Switzerland[b]; Federal Veterinary Office, Bern, Switzerland[c]

Multilocus sequence typing (MLST) and antibiotic resistance patterns of *Campylobacter jejuni* and *Campylobacter coli* from retail chicken meat showed high overlap with isolates collected at slaughterhouses, indicating little selection along the production chain. They also showed significant common sequence types with human clinical isolates, revealing chicken meat as a likely source for human infection.

Campylobacteriosis remains the most frequently reported zoonosis in the European Union and in Switzerland (1, 2). Most studies found consumption of poultry meat to be the main risk factor associated with human disease (3, 4, 5). While there are several studies describing the genotypes of *Campylobacter jejuni* and *Campylobacter coli* isolates from chickens at slaughter, only a few, and none so far in Switzerland, have looked at the genotypes of isolates from retail meat.

To study the diversity of isolates from chicken retail meat and their relation to isolates from chickens at slaughter, we investigated 204 *C. jejuni* and *C. coli* isolates that had been collected at the retail level in the course of a nationwide prevalence study in 2009 (6). Thereof, 141 isolates originated from Swiss meat and 57 originated from imported meat. For six isolates, the information on origin was unknown. For comparison with chickens at slaughter, 197 isolates from cecal samples, recovered in the context of the Swiss Antibiotic Resistance Monitoring Program 2009, were available. In addition, we compared the isolates from both groups to a set of 383 previously published isolates from diseased humans with no history of foreign travel that had been sampled in the same year (7).

Multilocus sequence typing (MLST) and genetic determination of resistance to quinolones and macrolides were performed as previously described (7, 8). Descriptive statistical analyses as well as Fisher's exact test and a chi-square test were conducted with the NCSS 8 software (NCSS, Kaysville, UT), setting the level of significance at P values of <0.05. The proportion similarity index (PSI) as a measure of the area of intersection between two frequency distributions was calculated, and the standard deviation for the estimation of confidence intervals was determined using the bootstrap method with 1,000 resampling steps (9, 10). Fixation index values (FST), which give an indication of the genetic distance between populations with a range from 0 to 1, were obtained with the Arlequin software version 3.5.1.2 (11), which was also used to perform analyses of molecular variance (AMOVA). FST and AMOVA were calculated using the concatenated sequences of the seven MLST alleles.

Species distribution was highly similar between isolates from retail (69% *C. jejuni*, 31% *C. coli*) and slaughterhouse (74% *C. jejuni*, 26% *C.* coli) and different in comparison to clinical isolates (91% *C. jejuni*, 9% *C. coli*). These findings indicate that the lower frequency of *C. coli* in human patients is not due to decreased survival along the production chain but more likely a difference in the potential to cause disease compared to *C. jejuni*. Genotypic diversity was markedly higher among retail meat isolates than among isolates from slaughterhouses, a fact which can be explained by a substantial proportion (29%) of the meat being imported (Table 1). Overall, *C. jejuni* isolated from retail meat could be attributed to 42 different sequence types (STs) belonging to 16 clonal complexes (CCs) and seven STs without a defined CC. When only Swiss meat was considered, this number dropped to 30 STs belonging to 15 CCs and four STs without a defined CC. The three most frequent CCs present in Swiss meat were CC21 (35%), CC48 (14%), and CC45 (11%), also found in similar proportions among the slaughterhouse isolates: CC21, 30%; CC48, 13%; CC45, 8%. The genetic diversity of the *C. jejuni* isolates from the slaughterhouses was also similar to that of the Swiss meat isolates, with 22 STs belonging to 14 CCs and 10 STs for which no CC was defined. No significant difference (chi-square test) was detected for the distribution of CCs between Swiss meat and slaughterhouse isolates, and the PSI was 0.85 (95% confidence interval [CI], 0.76 to 0.94). When STs were compared, there was also no significant difference between the two groups (chi-square test), and the PSI was still 0.69 (95% CI, 0.60 to 0.78), indicating that little selection takes place along the production chain.

C. coli isolates were less diverse than *C. jejuni* isolates, with 89% of the meat isolates and 85% of the slaughterhouse isolates belonging to CC828 and no CC defined for the rest. Two of the three most frequent STs (ST827, ST854) were the same for Swiss meat and slaughterhouse isolates. The PSI between these two groups amounted to 0.60 (95% CI, 0.46 to 0.74).

FST values between the slaughterhouse and Swiss retail meat isolates were not significantly different from zero for both species, indicating that essentially the same strains colonizing poultry at

Received 12 February 2013 **Accepted** 4 April 2013
Published ahead of print 12 April 2013
Address correspondence to Peter Kuhnert, peter.kuhnert@vetsuisse.unibe.ch.

doi:10.1128/AEM.00493-13

TABLE 1 Distribution of CCs and STs among *C. jejuni* and *C. coli* isolates from retail chicken meat and Swiss chicken at slaughter[a]

Species	CC	ST	No. of total isolates (no. of isolates from Swiss meat)	
			Retail	Slaughterhouse
C. jejuni	21	19	5 (5)	2
	21	21	17 (14)	18
	21	50	12 (8)	13
	21	104	1 (0)	0
	21	262	6 (6)	11
	21	597	1 (1)	0
	21	883	2 (0)	0
	22	22	3 (2)	5
	42	42	1 (1)	0
	42	469	1 (0)	0
	42	4765	1 (0)	0
	45	45	6 (4)	9
	45	137	5 (4)	0
	45	418	1 (1)	0
	45	583	1 (0)	1
	45	845	0 (0)	2
	45	1964	1 (1)	0
	45	**5125**	1 (1)	0
	48	48	13 (13)	19
	48	475	2 (1)	0
	52	52	1 (1)	0
	61	61	0 (0)	1
	206	122	2 (1)	7
	206	227	1 (1)	0
	206	290	1 (1)	0
	206	572	1 (0)	0
	206	2282	2 (1)	0
	206	3335	1 (0)	0
	257	257	4 (3)	10
	257	824	1 (0)	0
	257	990	0 (0)	1
	283	267	6 (4)	2
	283	383	4 (3)	4
	353	356	1 (1)	0
	353	5116	0 (0)	1
	354	354	2 (0)	0
	354	878	3 (3)	5
	354	1073	3 (1)	4
	354	1723	1 (0)	0
	354	2288	1 (0)	0
	443	51	5 (3)	0
	446	978	1 (1)	0
	460	460	1 (0)	0
	464	464	3 (3)	7
	607	607	3 (3)	0
	607	3963	3 (3)	6
	658	1044	0 (0)	1
	692	991	0 (0)	1
	ND	441	2 (1)	1
	ND	586	3 (3)	5
	ND	791	0 (0)	1
	ND	877	1 (0)	0
	ND	905	1 (0)	0
	ND	1080	0 (0)	2
	ND	1911	1 (1)	1
	ND	3544	1 (0)	0
	ND	4871	0 (0)	1
	ND	5115	0 (0)	1
	ND	5117	0 (0)	1
	ND	5118	0 (0)	1
	ND	5119	0 (0)	1
	ND	5124	1 (1)	0
Total			141 (101)	145
C. coli	828	825	3 (2)	8
	828	827	14 (13)	12
	828	828	1 (0)	0
	828	829	6 (6)	1
	828	854	6 (4)	5
	828	855	1 (0)	0
	828	1016	1 (0)	1
	828	1058	1 (0)	0
	828	1096	0 (0)	3
	828	1181	1 (0)	0
	828	1556	3 (3)	3
	828	1563	1 (1)	0
	828	1585	1 (0)	0
	828	1614	2 (0)	0
	828	1628	4 (0)	0
	828	1957	1 (1)	2
	828	2142	1 (1)	1
	828	2273	2 (2)	1
	828	2301	1 (0)	0
	828	3023	2 (2)	1
	828	3083	1 (0)	0
	828	3336	0 (0)	1
	828	3339	0 (0)	1
	828	3985	1 (0)	0
	828	4956	0 (0)	1
	828	5120	0 (0)	1
	828	5122	0 (0)	1
	828	5123	0 (0)	1
	828	**5126**	1 (0)	0
	828	**5127**	1 (1)	0
	ND	1581	1 (0)	0
	ND	1584	2 (2)	2
	ND	3344	2 (1)	2
	ND	3345	0 (0)	1
	ND	3989	1 (1)	0
	ND	4808	1 (0)	0
	ND	4936	0 (0)	1
	ND	4962	0 (0)	1
	ND	5121	0 (0)	1
Total			63 (40)	52

[a] New STs found in this study are indicated in bold. ND means no CC defined for this ST.

slaughter can also be found on the end product at retail. This observation is supported by a recent study from the United Kingdom where the predominant strains in poultry flocks were also the predominant strains found on the final carcass (12), but it is in contrast to other studies where differential survival of genotypes was observed (13, 14).

When isolates from Swiss and imported meat were compared, results differed for *C. jejuni* and *C. coli*. For *C. jejuni*, the FST between the two groups was not significantly different from zero. However, when performing locus-by-locus AMOVA, separating

TABLE 2 Frequency of genotypic macrolide resistance among *C. coli* isolates from retail chicken meat, Swiss chicken at slaughter, and human domestic cases (7)

Source	% (95% CI) of *C. coli* isolates resistant to macrolides
Retail meat (total)	8 (3–18)
Retail meat (Swiss)	5 (0–17)
Retail meat (import)	10 (0–30)
Chicken at slaughter	6 (0–16)
Humans (domestic cases)	0 (0–11)

TABLE 3 Frequency of genotypic quinolone resistance among *C. jejuni* and *C. coli* isolates from retail chicken meat, Swiss chicken at slaughter, and human domestic cases (7)

Source	% (95% CI) of isolates resistant to quinolones	
	C. jejuni	*C. coli*
Retail meat (total)	34 (26–42)	59 (46–71)
Retail meat (Swiss)	27 (18–36)	50 (34–66)
Retail meat (import)	53 (35–69)	71 (48–89)
Chicken at slaughter	29 (22–37)	40 (27–55)
Humans (domestic cases)	39 (34–45)	38 (21–56)

isolates by quinolone resistance and then by origin (Swiss or imported), a significant amount of variation was assigned to the quinolone resistance variable (4.2%), while origin accounted only for 1.7%. For *C. coli*, the FST between isolates from Swiss and imported meat amounted to 0.084, which was a significant difference. Interestingly, when locus-by-locus AMOVA was performed for *C. coli* as described above, no significant amount of variation was assigned to quinolone resistance, whereas 10.9% was assigned to origin (Swiss or imported). This indicates that *C. jejuni* genotypes are largely similar between Swiss and imported meat, while a difference could be detected for *C. coli* genotypes. On the other hand, there seems to be a nonrandom association of some *C. jejuni* genotypes with quinolone resistance independent of the retail meat's origin.

Compared to the human isolates, taking *C. jejuni* and *C. coli* together, both the retail (including Swiss and imported meat) and the slaughterhouse group showed a high overlap, with a PSI of 0.51 (95% CI, 0.44 to 0.57) and 0.53 (95% CI, 0.46 to 0.60), respectively. The FST for *C. jejuni* was 0.028 (95% CI of 0.024 to 0.034) when human isolates were compared to retail (including Swiss and imported meat) and 0.026 (95% CI, 0.023 to 0.031) when they were compared to the slaughterhouse isolates. The difference between human and poultry isolates was slightly but not significantly higher for *C. coli*, with FST values of 0.048 (95% CI, 0.002 to 0.085) and 0.044 (95% CI, 0.005 to 0.080), respectively. This finding might suggests that *C. coli* shows more host specificity, with some genotypes being more infectious for humans than others, but this conclusion must be drawn with caution due to the much smaller sample size for *C. coli*. However, the low FST in both cases indicates a strong association of chicken and human isolates.

There was no significant difference (Fisher's exact test) in antibiotic resistance between isolates from Swiss meat and slaughterhouses. The mutation conferring macrolide resistance (23S RNA gene: A2075G) could not be demonstrated in *C. jejuni*, whereas in *C. coli*, 5% of the isolates from Swiss meat and 6% of the slaughterhouse isolates possessed this mutation (Table 2). Quinolone resistance (mutation C257T or A in *gyrA*) was also more frequent among *C. coli* occurring in 50% of the Swiss meat and 40% of the slaughterhouse isolates (Table 3). Among *C. jejuni* isolates, the respective frequencies were only 27% and 29%. In contrast, *C. jejuni* from imported meat showed a significantly (Fisher's exact test) higher occurrence of quinolone resistance (53%). *C. coli* from imported meat also displayed a tendency (not significant) toward higher quinolone and macrolide resistance than isolates from Swiss meat (Table 2 and 3). Interpretation of these numbers is difficult, as the countries of origin for imported meat could not be determined. However, the resistance situation appears to be better in Switzerland than in many other European countries (15). Nevertheless, a steady increase in quinolone resistance has been observed among *C. jejuni* isolates from Swiss chickens over the last years (16). This is not surprising, as enrofloxacin is the most frequently applied antibiotic on Swiss broiler farms (17).

In conclusion, isolates from chickens at slaughter are highly congruent with isolates from Swiss meat at retail, indicating that little selection of genotypes seems to take place along the production chain. Also, a strong genotypic relationship of *Campylobacter* isolates from broiler meat and human cases was shown. These data give further evidence that contaminated poultry meat is the most important risk factor for human campylobacteriosis, and they help to strengthen the scientific background for concrete risk management measures in the framework of the ongoing campylobacteriosis epidemics in Switzerland as well as other countries.

ACKNOWLEDGMENT

This work was supported by Swiss Federal Veterinary Office grant 1.10.08.

REFERENCES

1. **Anonymous.** 2011. Schweizer Zoonosebericht 2010. Bundesamt für Veterinärwesen BVet. FVO, Bern, Switzerland. http://www.bvet.admin.ch.
2. **European Food Safety Authority.** 2011. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J. **9**:2090.
3. **Büttner S, Wieland B, Stärk KD, Regula G.** 2010. Risk attribution of *Campylobacter* infection by age group using exposure modelling. Epidemiol. Infect. **138**:1748–1761.
4. **Neimann J, Engberg J, Molbak K, Wegener HC.** 2003. A case-control study of risk factors for sporadic *Campylobacter* infections in Denmark. Epidemiol. Infect. **130**:353–366.
5. **Wingstrand A, Neimann J, Engberg J, Nielsen EM, Gerner-Smidt P, Wegener HC, Molbak K.** 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. Emerg. Infect. Dis. **12**:280–285.
6. **Baumgartner A, Felleisen R.** 2011. Market surveillance for contamination with thermotolerant campylobacters on various categories of chicken meat in Switzerland. J. Food Prot. **74**:2048–2054.
7. **Niederer L, Kuhnert P, Egger R, Buttner S, Hachler H, Korczak BM.** 2012. Genotypes and antibiotic resistances of *Campylobacter jejuni* and *Campylobacter coli* isolates from domestic and travel-associated human cases. Appl. Environ. Microbiol. **78**:288–291.
8. **Korczak BM, Zurfluh M, Emler S, Kuhn-Oertli J, Kuhnert P.** 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.
9. **Rosef O, Kapperud G, Lauwers S, Gondrosen B.** 1985. Serotyping of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter laridis* from domestic and wild animals. Appl. Environ. Microbiol. **49**:1507–1510.
10. **Müllner P, Spencer SE, Wilson DJ, Jones G, Noble AD, Midwinter AC, Collins-Emerson JM, Carter P, Hathaway S, French NP.** 2009. Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach. Infect. Genet. Evol. **9**:1311–1319.
11. **Excoffier L, Lischer HEL.** 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. **10**:564–567.

12. **Elvers KT, Morris VK, Newell DG, Allen VM.** 2011. Molecular tracking, through processing, of *Campylobacter* strains colonizing broiler flocks. Appl. Environ. Microbiol. **77**:5722–5729.
13. **Colles FM, McCarthy ND, Sheppard SK, Layton R, Maiden MC.** 2010. Comparison of *Campylobacter* populations isolated from a free-range broiler flock before and after slaughter. Int. J. Food Microbiol. **137**:259–264.
14. **Hunter SM, Berrang ME, Meinersmann RJ, Harrison MA.** 2009. Genetic diversity of *Campylobacter* on broiler carcasses collected preevisceration and postchill in 17 US poultry processing plants. J. Food Prot. **72**:49–54.
15. **European Food Safety Authority.** 2012. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2010. EFSA J. **10**:2598.
16. **Büttner S, Flechtner O, Müntener C, Overesch G.** 2012. Bericht über den vertrieb von antibiotika in der veterinärmedizin und das antibiotikaresistenzmonitoring bei nutztieren in der schweiz (ARCH-VET 2011) bundesamt für veterinärwesen und swissmedic. FVO, Bern, Switzerland. http://www.swissmedic.ch.
17. **Büttner S, Kuhn M.** 2010. Antibiotikaresistenzmonitoring 2008. FVO, Bern, Switzerland. http://www.bvet.admin.ch.