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# Genotypes and Antibiotic Resistances of *Campylobacter jejuni* and *Campylobacter coli* Isolates from Domestic and Travel-Associated Human Cases

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**Multilocus sequence typing (MLST) extended with *flaB* typing of 425 *Campylobacter jejuni* isolates and 42 *Campylobacter coli* isolates revealed quite a low overlap between human isolates from travel-associated and domestic cases in Switzerland. Men were more frequently affected by *Campylobacter* than women, but strains from women and, overall, from travel-associated cases showed mutations conferring quinolone resistance more frequently than strains from men and domestic cases, respectively.**

*Campylobacter jejuni* and *Campylobacter coli* are the major causes of bacterial gastroenteritis worldwide. In Switzerland, the reported incidence is one of the highest in Europe (3, 4, 9, 21, 23). The infection occurs mainly after the handling of raw meat and the consumption of undercooked contaminated meat, but traveling abroad has also been recognized as a risk factor (9, 20–22). To gain information on genotypes and antibiotic resistances of strains implicated in either domestic or travel-associated cases, 425 *C. jejuni* isolates and 42 *C. coli* isolates representing 8.2% of all reported cases in the sampling period from June to December 2009 in Switzerland were investigated (Table 1). Most of the isolates (383; 82.0%) were obtained from domestic cases. Taking into consideration the incubation time of *Campylobacter* spp. and how long clinical symptoms last, the remaining 84 isolates (18.0%) from patients who had been abroad within the last 2 weeks prior to medical consultation were classified as travel associated (4). This is quite a low rate in comparison to that of an earlier case-control study conducted in Switzerland, where 46.1% of investigated cases were associated with foreign travel (22). The distribution of isolates within the particular age groups of patients was highly reflected in the proportion calculated for all reported cases in Switzerland in 2009 (<http://www.bag.admin.ch>), underlining the representativeness of the spot sample.

A significantly higher number of patients were male (267; 57.2%), as found in other studies (11, 12, 21). The reason for this dimorphism in campylobacteriosis is yet unknown, but it has been speculated that there are sexual differences concerning handling and consumption of meat (21). However, Strachan et al. (25) challenged this view by showing similar differences in rates of campylobacteriosis even in children younger than 1 year old, in whom behaviors are expected to be similar regardless of gender. Moreover, these authors observed similarly different predispositions toward campylobacteriosis based on gender when male and female mice were infected with *C. jejuni*.

In terms of age (Table 1), patients in the travel group were significantly younger (median, 30 years) than those of the domestic group (median, 38 years) ( $P = 0.021$ ).

The distribution of species (91.0% *C. jejuni* and 9.0% *C. coli*) reflected the proportion found in other studies from various countries and confirms *C. jejuni* as the main causative agent of campylobacteriosis (11, 23). There was no association between

*Campylobacter* species and age, gender, or travel history. This contrasts with an English study which described a dependence between *C. coli* and female and older patients (11).

Multilocus sequence typing (MLST), *flaB* typing, and genetic determination of resistance to quinolones and macrolides, the two main groups of antibiotics used for treatment of severe cases, based on the sequencing of *gyrA* and 23S rRNA gene fragments, respectively, were performed as described previously (2, 5, 6, 17, 19). In contrast to the previously described protocol (17), 5× FIREPol master mix ready to load (Solis BioDyne, Tartu, Estonia) was used. This led to the following composition for each 30.0- $\mu$ l multiplex PCR: 0.4  $\mu$ M appropriate primer, 1× FIREPol master mix ready to load, 1.0  $\mu$ l lysate (approximately 30 ng DNA), and the addition of MgCl<sub>2</sub> up to a final concentration of 5 mM. Data analysis was performed as previously described by Kittl et al. (16).

MLST resulted in 111 sequence types (STs), and *flaB* typing resulted in 87 types; 17 of each were new (Table 2). The *C. jejuni* isolates showed much higher diversity than the *C. coli* isolates, as indicated by the distribution of the strains within different clonal complexes (CCs). Of the investigated *C. coli* strains, 95.0% could be assigned to CC828, confirming a higher clonality within this species (17, 24, 26).

Despite the many CCs within the *C. jejuni* strains, the majority of strains (62.0%) could be assigned to the three most common CCs, i.e., CC21, CC48, and CC257. These lineages were frequently found in human samples described in other studies from different countries (6, 18, 23). This suggests that they might be the best adapted or most pathogenic for humans. Interestingly, as previously described (16), less than 2.0% of *C. jejuni* isolates belonged to CC45, which is the most common CC found in humans in most countries and especially in Finland (15, 23). As poultry is supposed to be the main source of campylobacteriosis, the number of human isolates belonging to CC45 is remarkably low even though

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TABLE 1 Number of isolates according to age and gender of patients<sup>a</sup>

Patient age (yr)	No. of isolates				Total no. of isolates
	Female		Male		
	Domestic case	Travel-associated case	Domestic case	Travel-associated case	
0–9	13 (6)	2 (1)	16 (2)	7 (3)	38 (12)
10–19	17 (6)	5 (2)	18 (5)	5 (4)	45 (17)
20–29	39 (24)	11 (9)	48 (16)	10 (5)	108 (54)
30–39	27 (12)	6 (4)	26 (9)	13 (6)	72 (31)
40–49	23 (11)	3 (1)	40 (18)	5 (2)	71 (32)
50–59	14 (7)	1 (1)	24 (10)	9 (5)	48 (23)
60–69	14 (5)	1 (0)	26 (9)	4 (3)	45 (17)
70–79	14 (6)	2 (1)	10 (2)	0 (0)	26 (9)
≥80	6 (0)	0 (0)	4 (2)	0 (0)	10 (2)
Unknown	2 (1)	0 (0)	2 (0)	0 (0)	4 (1)
Total	169 (78)	31 (19)	214 (73)	53 (28)	467 (198)

<sup>a</sup> Numbers of quinolone-resistant isolates are in parentheses.

it was one of the most frequent CCs found in Swiss broilers (28). There is no obvious explanation for this, and further investigation is needed.

As with previous investigations, *flaB* typing was poorly congruent with MLST (17, 28). Thus, a combination of MLST and *flaB* typing increased discrimination, as reflected in a higher Simpson's index of diversity: 0.970 compared to 0.928 for MLST and 0.921 for *flaB* typing alone. This provided appropriate information for the study of *Campylobacter* epidemiology.

Only 25 out of 65 combined MLST-*flaB* genotypes, found in the group of travel-associated cases and represented by 51.2% of isolates, matched those of the isolates obtained from domestic cases. The existing overlap of these two groups was due mainly to ST-21, ST-50, and ST-48 combined with *flaB* type 103, which were represented in both of them and which are the most frequent STs worldwide (15, 16, 18). Conversely, the new STs found among 2.6% of domestic strains, together with ST-3963, which was first described in isolates from Swiss broilers in 2008 and which also appeared among the domestic isolates, might represent clones that are endemic to Switzerland. The difference in population structures of the two groups of isolates was confirmed by a low proportional similarity index (PSI, 0.35) (10).

A significantly higher number of quinolone-resistant strains was found among travel-associated cases (56.0%) than among domestic cases (39.4%) (Table 2). Foreign travel as a risk factor for infection with quinolone-resistant *Campylobacter* species strains had been described previously, but the reason for this remains unknown (14). The rates of quinolone-resistant strains in humans in different countries range from 0% to over 80%, and this variation could be ascribed to the divergent use of quinolones in food-producing animals (8). A correlation between licensing quinolone use in food animals and the appearance of resistant strains in humans has been shown previously (1).

There was no significant difference in the rates of quinolone resistance between strains of domestic origin and those from patients who have traveled to member countries of the European Union. This could be due to similar regulations in Switzerland and European Union member countries, because both ban the use of antibiotics as feed additives (8). In contrast, a considerably higher rate of quinolone resistance was observed in strains acquired in

Asia, consistent with previous reports (7, 14). In addition, travelers to Asia and Latin America often take preventive antibiotics, such as quinolones (7).

A high number of resistant strains in the group of travel-associated cases belonged to STs which did not occur in the group of domestic cases. Strains with the common STs found in both groups showed no significant difference in antibiotic resistance.

Most strains resistant to quinolones (88.4%) possessed the C257T transition in the *gyrA* gene (Table 3); this is the most frequent mutation in *C. jejuni* and *C. coli* (2). Interestingly, we found a strong association between the double mutation C257T C310A and the domestic isolates representing ST-50 and *flaB* type 103, data in support of a clone endemic to Switzerland. At position 310, a substitution of serine (TCA) for proline (CCA), conferring resistance to quinolones, is normally observed (2). The effect on resistance of the transversion from CCA (proline) to ACA (threonine) found in our strains could not be tested because of the presence of the C257T mutation, which singly confers quinolone resistance.

The only isolate resistant to macrolides was also phenotypically resistant to quinolones. Remarkably, it possessed a novel double mutation within the *gyrA* triplet coding for Thr86 that led to Thr86Val (GenBank accession no. JN815082). This strain was isolated from a patient who had traveled to Bali, and it had probably undergone different selection pressures than the other isolates.

The analysis of quinolone resistance with respect to gender revealed a significantly higher percentage in women (48.7%) than in men (38.4%) ( $P = 0.029$ ), a finding, to our knowledge, not described before. There might be a connection with urinary tract infections, which are more frequently diagnosed in women than in men and, in many cases, treated with quinolones (13). This might lead to the secondary acquisition of posttherapeutic resistance to this class of antibiotics in *Campylobacter*. In contrast to another study which described higher rates of quinolone resistance in *C. coli* (27), we found no significant difference in antibiotic resistance rates between *C. jejuni* and *C. coli*.

In conclusion, there are differences between Swiss domestic and travel-associated *Campylobacter* isolates with respect to genotype and quinolone resistance.

TABLE 2 Distribution of CCs, STs, and *flaB* types among *C. jejuni* and *C. coli* strains isolated from humans in 2009<sup>a</sup>

Species	CC	ST	<i>flaB</i> type	No. of domestic cases	No. of travel-associated cases	Species	CC	ST	<i>flaB</i> type	No. of domestic cases	No. of travel-associated cases			
<i>C. jejuni</i>	21	8	<b>1525</b>		1 (0)			4370	103	1 (1)				
		19	36	7 (6)				4370	<b>1535</b>	1 (1)				
		19	53	2 (0)				<b>5108</b>	<b>1530</b>					1 (1)
		21	8	1 (1)						354		354	18	
		21	51	1 (0)					354	222				1 (0)
		21	103	32 (11)				8 (2)	878	34			4 (3)	
		21	105	1 (0)					1073	208			5 (1)	1 (0)
		21	198	35 (20)				4 (3)	<b>5098</b>	34			2 (2)	
		21	226	3 (0)					<b>5105</b>	18				1 (1)
		21	371	1 (0)					403	403		51		1 (0)
		21	414	9 (0)					1775	51				1 (0)
		21	487	1 (1)					443	51		21	8 (6)	1 (1)
		21	<b>1520</b>	1 (0)					446	446		222	1 (1)	1 (1)
		21	<b>1526</b>		1 (0)					862		222	1 (0)	
		21	<b>1529</b>	1 (1)						2850		18	1 (1)	
		50	36	16 (1)				4 (1)	460	989		34	1 (1)	
		50	103	20 (20)				1 (0)		4701		34		1 (0)
		50	350	3 (0)						<b>5106</b>		34		1 (1)
		50	431	2 (2)					574	2031		112	1 (1)	
		50	1246	1 (1)				1 (1)		4057		402	1 (1)	
		262	137	11 (0)				1 (0)	607	607		14		1 (1)
	883	103	1 (0)				3963	103	6 (0)					
	883	1386		1 (1)			3963	<b>1519</b>	1 (0)					
	917	96	2 (0)			658	657	5	2 (1)					
	1519	34	1 (1)				1044	5		1 (1)				
	1943	138	1 (1)				1900	117	1 (0)	1 (1)				
	2135	260		1 (1)		677	677	864	5 (0)					
	3102	34		2 (2)		1034	1709	18	1 (0)					
	<b>5104</b>	198	2 (2)				1709	<b>1528</b>	1 (0)					
	<b>5107</b>	103	1 (1)			ND	441	34	4 (4)					
	22	22	309	1 (0)			441	198		1 (1)				
		22	442	5 (1)	1 (0)		448	<b>1514</b>	1 (0)					
		22	<b>1516</b>		1 (0)		449	612	1 (1)					
		<b>5103</b>	442	1 (0)			464	34	1 (1)					
	42	42	177	1 (0)	1 (0)		464	45	3 (3)					
	45	45	15	1 (0)			464	103	2 (2)					
		45	21	1 (0)			464	<b>1522</b>	1 (1)					
		45	31	1 (0)			464	<b>1533</b>	2 (2)					
		45	307	1 (0)			531	100		1 (1)				
		137	16	1 (0)			586	402	2 (0)					
		418	307		1 (0)		881	67	1 (1)	1 (1)				
		583	177	1 (1)			1367	<b>1513</b>	2 (0)					
		755	22	1 (0)			1726	402		1 (1)				
	48	1964	31		1 (0)		2175	350	1 (0)					
		48	36		1 (0)		2274	112	1 (1)					
		48	103	32 (4)	3 (1)		2274	117		1 (1)				
		48	105	1 (0)			2274	1248	1 (1)	1 (1)				
		48	198	1 (0)			2332	54		1 (1)				
		475	105	1 (0)	1 (0)		3544	49	1 (1)					
		2309	103	1 (1)			4373	208	1 (1)					
	<b>5109</b>	103	1 (0)			4800	67	1 (1)						
49	49	11		1 (0)		<b>5100</b>	16		1 (1 <sup>b</sup> )					
52	52	57	2 (1)	3 (2)		<b>5101</b>	<b>1518</b>	1 (0)						
		<b>5110</b>	57	1 (0)		<b>5102</b>	260	1 (1)						
61	61	44	1 (0)		Total			351 (139)	74 (40)					
	61	1179	3 (0)		<i>C. coli</i>	828	825	16	3 (2)					
206	61	1209	1 (0)			827	66		1 (0)					
	122	47	4 (0)			827	103	1 (0)						
	122	395	3 (1)	1 (1)		827	236	14 (4)	1 (0)					
	227	47	1 (1)	1 (1)		827	556	1 (1)						
	572	47	2 (2)			854	915	1 (0)						
	572	105	1 (0)			855	66	1 (0)						
	572	198	1 (1)	1 (1)		872	16	1 (0)						
	572	1384		1 (1)		872	30		1 (1)					
	3335	96	1 (1)			899	13	1 (1)						
	<b>5099</b>	986		1 (1)		1145	494	1 (1)						
257	257	16	19 (1)	2 (1)		1173	765	2 (2)						
	257	301	13 (3)	1 (1)		1191	16		1 (0)					
	367	16		1 (1)		1413	<b>1524</b>	1 (0)						
	824	34	1 (1)	1 (0)		2142	66	1 (0)	1 (1)					
	2254	16	1 (1)			2273	1117	1 (0)						
283	267	177		1 (0)		3336	<b>1517</b>	1 (0)						
	383	177	3 (0)			3990	30		1 (1)					
353	5	96	3 (3)			<b>5111</b>	17		1 (1)					
	353	45	3 (0)	1 (1)		<b>5112</b>	125	1 (0)						
	356	114	1 (0)			<b>5113</b>	13		1 (1)					
	400	67	1 (0)			<b>5114</b>	<b>1532</b>		1 (1)					
	2801	320	1 (1)		ND	1143	824	1 (1)						
	2849	96	2 (2)			1592	836		1 (1)					
	2882	96	1 (1)		Total			32 (12)	10 (7)					
	4370	67	2 (2)											

<sup>a</sup> The numbers of quinolone-resistant isolates are in parentheses. New STs and/or new *flaB* types are in bold. ND, not defined.

<sup>b</sup> Strain resistant to quinolone and macrolide.

TABLE 3 Positions of mutations within *gyrA*, conferring quinolone resistance

No. of isolates	Position of mutations within <i>gyrA</i>					
	A256G C257T (Thr86Val) <sup>a</sup>	C257T (Thr86Ile)	C257A (Thr86Lys)	G268A (Asp90Asn)	G268C (Asp90His) <sup>b</sup>	C310A (Pro104Thr) <sup>b</sup>
175		x				
1			x			
19		x				x
1		x			x	x
1		x		x		
1	x					

<sup>a</sup> New variant of *gyrA* in a macrolide- and quinolone-resistant *C. jejuni* strain.

<sup>b</sup> Observed mutations which are different from known resistance-conferring ones.

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