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Characterization of *C. jejuni* and *C. coli* broiler isolates by whole genome sequencing

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Introduction

Campylobacter is the most commonly reported cause of bacterial diarrhoeal disease in humans in the EU since 2005. The major source of infection is contaminated poultry meat with most broiler batches at slaughter colonized with *Campylobacter*. *C. jejuni* and *C. coli* are responsible for the vast majority of infections, which may subsequently lead to serious neuropathologies such as Guillain-Barré syndrome.

The **aim** of this study was to take advantage of whole genome sequencing (WGS) to in-depth characterize a subset of 16 *C. jejuni* and *C. coli* isolates from broilers from five farms.

Results

- A total of 8420 SNPs were identified and used to perform the **phylogenetic analysis** (**Figure 1**).
- All isolates were positive for almost all of the **34 virulence-associated genes** (**Table 1**).
- All the strains showed a **multidrug resistant** profile (**Table 2**).

Table 1. Identification of virulence-associated genes in *C. jejuni* and *C. coli* isolates.*

Isolates[a]	Species	Motility		GBS[b]	Hippuricase	Multidrug and bile resistance			Iron uptake	
		flaA	*flaB*	*wlaN*	*hipO*	*cmeA*	*cmeB*	*cmeC*	*cfrA*	*fur*
A1	*C. coli*	+	+	–	–	+	+	+	+	+
A2	*C. jejuni*	–	+	–	+	+	+	+	+	+
B3	*C. jejuni*	+	+	–	+	+	+	+	+	+
B4	*C. jejuni*	+	+	–	+	+	+	+	+	+
B5	*C. jejuni*	+	+	–	+	+	+	+	+	+
B6	*C. jejuni*	–	+	–	+	+	+	+	+	+
C7	*C. coli*	+	+	–	–	+	+	+	+	+
C8	*C. coli*	+	+	–	–	+	+	+	+	+
C9	*C. jejuni*	+	+	+	+	+	+	+	+	+
C10	*C. jejuni*	–	+	–	+	+	+	+	+	+
D11	*C. jejuni*	+	+	–	+	+	+	+	+	+
D12	*C. jejuni*	–	+	–	+	+	+	+	+	+
E13	*C. jejuni*	+	+	+	+	+	+	+	+	+
E14	*C. jejuni*	–	+	–	+	+	–	+	+	+
E15	*C. jejuni*	+	+	–	+	+	+	+	–	+
E16	*C. coli*	+	+	–	–	+	+	+	+	+
Total		11	16	2	12	16	15	16	15	16

* The presence of genes related to motility (*flhA, flhB, flgB, flgE, fliM, fliY*); chemotaxis (*cheA, cheB, cheR, cheW, cheY*); adhesion (*cadF, dnaJ, pdlA, racR*); capsule (*kpsM, waaF*); invasion (*iamA, ciaB, ceuE*); cytolethal distending toxin (*cdta, cdtB, cdtC*); stress response and survival (*katA, sodB*), was also confirmed in all the isolates, (data not shown to facilitate the reading). [a] Isolates from farm A, B, C, D and E. [b] GBS, Guillain-Barré syndrome.

Materials & Methods

FARM	A	B	C	D	E	Total isolates
C. jejuni	1	4	2	2	3	12
C. coli	1	0	2	0	1	4
Total	**2**	**4**	**4**	**2**	**4**	**16**

Next-Generation Sequencing (Illumina MiSeq paired-end)

MICs

De-novo assembly (**Assembler v1.2[#]**)

SNPs analysis (snpTree v1.1[#])

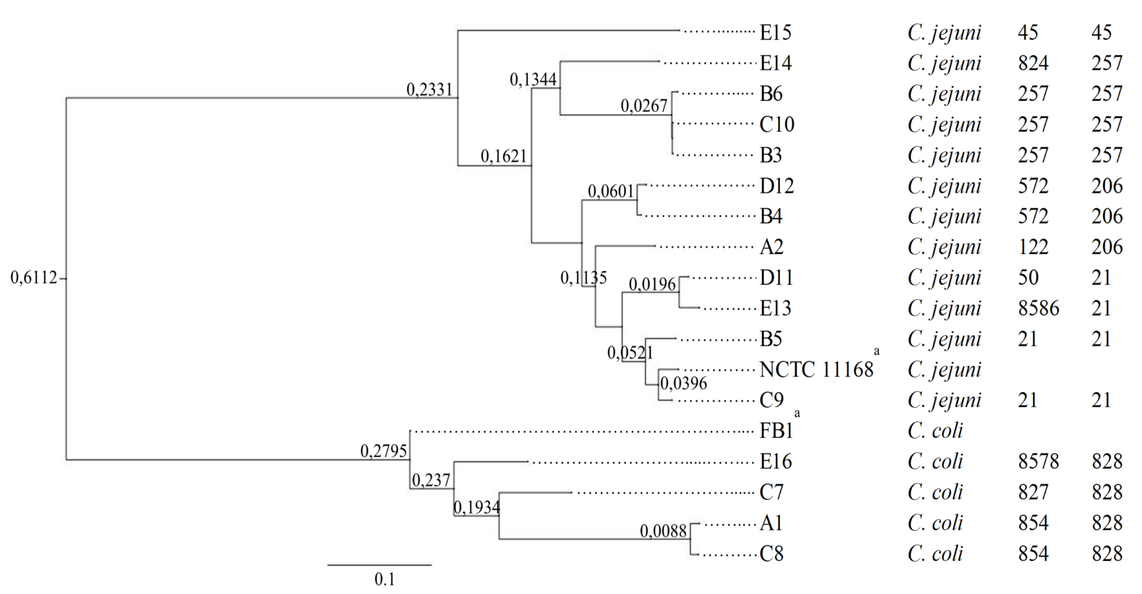
MLST analysis (MLST v1.8[#])

Virulence genes (MyDbFinder v1.1[#])

Resistance genes (ResFinder v2.1[#])

[#] Web tools are available at Center for Genomic Epidemiology (**https://cge.cbs.dtu.dk/services/**)

Figure 1. Maximun-likelihood phylogenetic tree based on SNPs and genotypic MLST types of *Campylobacter* isolates.



[a] Reference genomes for *C. jejuni* NCTC 11168 and for *C. coli* FB1, were included in the analysis. [b] ST, sequence type; CC, clonal complex

Table 2. Antimicrobial susceptibility (MIC) and associated resistance mechanisms of *Campylobacter* isolates.

Isolates	Species	Quinolones			Tetracycline		Aminoglycosides				Macrolide		Efflux pump CmeA,B,C
		Nal[a]	Ci	R-mech[c] Thr86Ile	Tc	R-mech *tet(O)*	Sm	R-mech *aphA(3´)*	R-mech *aadE*	Gm	R-mech aphA(3´)	Ery	R-mech 23S rDNA	
A1	*C. coli*	R (> 64)[b]	R (> 8)	+	S (0,5)	–	R (32)	+	–	R (2)	+	R (32)	+	+ + +
A2	*C. jejuni*	R (32)	R (> 8)	+	S (0,5)	–	S (0,5)	–	–	S (0,12)	–	R (32)	–	+ + +
B3	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (32)	–	–	S (0,12)	–	R (> 64)	–	+ + +
B4	*C. jejuni*	R (32)	R (0,5)	+	R (> 16)	+	R (8)	–	–	S (0,12)	–	S (1)	–	+ + +
B5	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (> 64)	+	+	R (2)	+	S (0,5)	–	+ + +
B6	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (> 64)	–	–	S (1)	–	S (1)	–	+ + +
C7	*C. coli*	R (> 64)	R (4)	+	R (2)	+	R (32)	–	–	S (0,12)	–	R (> 64)	+	+ + +
C8	*C. coli*	R (> 64)	R (4)	+	R (> 16)	+	R (> 64)	–	–	S (0,25)	–	R (> 64)	+	+ + +
C9	*C. jejuni*	R (32)	R (4)	+	R (> 16)	+	S (0,5)	–	–	S (0,12)	–	S (1)	–	+ + +
C10	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (> 64)	–	–	S (0,12)	–	R (> 64)	–	+ + +
D11	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	S (0,5)	–	–	S (0,12)	–	S (1)	–	+ + +
D12	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (> 64)	–	–	S (1)	–	R (> 64)	–	+ + +
E13	*C. jejuni*	R (> 64)	R (4)	+	R (> 16)	+	S (0,5)	–	–	S (0,12)	–	S (1)	–	+ + +
E14	*C. jejuni*	R (> 64)	R (> 8)	+	R (> 16)	+	R (> 64)	–	–	S (0,12)	–	R (8)	–	+ – +
E15	*C. jejuni*	R (> 64)	R (> 8)	+	S (0,5)	–	R (16)	–	–	S (0,25)	–	S (1)	–	+ + +
E16	*C. coli*	R (16)	R (> 8)	+	R (> 16)	+	R (4)	–	–	S (0,12)	–	R (> 64)	–	+ + +
Total		**100%**	**100%**		**81%**		**75%**			**13%**		**56%**		

[a] Nal: Nalidixic acid, Ci: Ciprofloxacin, Tc: Tetracycline, Sm: Streptomycin, Gm: Gentamicin and Ery: Erythromycin. [b] Interpretation of MIC values for *C. jejuni* epidemiological cut-off values: Nal (R ≥ 16 mg/L); Ci (R ≥ 0,5 mg/L); Tc (R ≥ 1 mg/L); Sm (R ≥ 4 mg/L); Gm (R ≥ 2 mg/L) and Ery (R ≥ 4 mg/L). Interpretation for *C. coli* epidemiological cut-off values: Nal (R ≥ 16 mg/L); Ci (R ≥ 0,5 mg/L); Tc (R ≥ 2 mg/L); Sm (R ≥ 4 mg/L); Gm (R ≥ 2 mg/L) and Ery (R ≥ 8 mg/L). [c] R-mech: resistance mechanism. Thr86Ile: point mutations in the subunit A of the DNA gyrase gene; *tet(O), aphA(3')* and *aadE*: presence of these genes; 23S rDNA: point mutation on this region of the genome.

Conclusions

- Two novel STs not previously reported in the PubMLST database were identified: **ST8586** (*C. jejuni*) and **ST8578** (*C. coli*).
- Phenotypic antimicrobial resistance obtained with the MIC analysis corresponded well for most of the isolates with the identification of specific antimicrobial resistance genes detected by WGS. The efflux pump, conferring resistance to a wide range of antimicrobials, was present in all but one isolate.
- WGS technology has become a fast and affordable tool and may become a rapid and cost-effective approach to characterize isolates from epidemiological studies.

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