**SHORT COMMUNICATION**

**Prevalence of Type VI Secretion System in Spanish Campylobacter jejuni Isolates**

M. Ugarte-Ruiz1,2, R. A. Stabler3, L. Domínguez1, M. C. Porrero1, B. W. Wren3, N. Dorrell3 and O. Gundogdu3

1 VISAVET Health Surveillance Centre, Universidad Complutense Madrid, Madrid, Spain
2 Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense Madrid, Madrid, Spain
3 Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK

**Impacts**

- Sixty three Spanish *C. jejuni* isolates (poultry and urban effluent) were investigated for presence of Type VI secretion system (T6SS) using whole-genome sequencing.
- The proportion of isolates harbouring all 13 T6SS ORFs was 14%.
- Further research would be necessary to determine the prevalence and importance of T6SS-positive *C. jejuni* strains.

**Summary**

Infections from *Campylobacter jejuni* pose a serious public health problem and are now considered the leading cause of foodborne bacterial gastroenteritis throughout the world. Sequencing of *C. jejuni* genomes has previously allowed a number of loci to be identified, which encode virulence factors that aid survival and pathogenicity. Recently, a Type VI secretion system (T6SS) consisting of 13 conserved genes was described in *C. jejuni* strains and recognised to promote pathogenicity and adaptation to the environment. In this study, we determined the presence of this T6SS in 63 Spanish *C. jejuni* isolates from the food chain and urban effluents using whole-genome sequencing. Our findings demonstrated that nine (14%) strains harboured the 13 ORFs found in prototype strain *C. jejuni* 108. Further studies will be necessary to determine the prevalence and importance of T6SS-positive *C. jejuni* strains.

**Introduction**

Campylobacteriosis is the most frequently reported zoonotic diarrhoeal disease worldwide with 80–90% of infections being attributed to *Campylobacter jejuni* (Humphrey et al., 2007; Fitzgerald et al., 2008; Epps et al., 2013; EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), 2014). Transmission of *Campylobacter* occurs throughout the food chain, often through cross-contamination (Epps et al., 2013). Consumption of poultry, beef and pork products is the leading cause of human foodborne illness, with poultry estimated to account for 50–70% of human *Campylobacter* infections (Jorgensen et al., 2002; Humphrey et al., 2007; Epps et al., 2013).

Recently, *C. jejuni* strains isolated from South-East Asia have been shown to harbour novel type VI secretion system (T6SS). T6SS are able to promote pathogenicity, symbiotic relationships and a selective adaptation to environmental perturbations (Jani and Cotter, 2010; Lerpriyapong et al., 2012). The *C. jejuni* T6SS was found to have pleiotropic effects ranging from virulence, influencing cell adhesion, cytotoxicity towards erythrocytes and colonisation of mice (Lerpriyapong et al., 2012; Bleumink-Pluym et al., 2013; Harrison et al., 2014). Current structural models of T6SS consist of a bacteriophage-like structure and a cell envelope-spanning membrane-associated assembly that translocates protein effectors into different cell types (Cascales and Cambillau, 2012; Silverman et al., 2012). A loci containing 13 ORFs can be subdivided into three groups; group one genes *tssJ*, *tssL* and *tssM* encode for membrane-associated proteins; group two genes *tssB*, *tssC*, *tssD* (*hcp*), *tssE* and *tssI* (*vgrG*) encode for proteins with function related to tailed bacteriophage components; group three genes *tssA*, *tssF*, *tssG*, *tssH* (*tagH*) and *tssK* encode for proteins with unknown function (Silverman et al., 2012; Fritsch et al., 2013).
Bleumink-Pluym et al., 2013 showed that of 80 investigated strains (which were predominantly from Europe or USA), only 10% harboured a T6SS. More recently a study compared the presence of T6SS in *C. jejuni* strains from the UK and Vietnam (Harrison et al., 2014), where T6SS was present in 60.6% and 71.4% of humans and chicken isolates respectively in Vietnam. However, in the UK, *C. jejuni* strains harbouring a T6SS from humans and chickens were noted as being 2.6% and 3.9%, respectively (Harrison et al., 2014). Given the difference between strains harbouring T6SS and the potential impact regarding strain virulence, we investigated the identification of T6SS loci within 63 Spanish *C. jejuni* strains isolated from poultry and urban effluents. The strains were isolated from a range of sources including faeces, neck skin, chicken meat and urban effluents. For the identification of T6SS in these *C. jejuni* isolates, whole-genome sequencing was performed.

**Materials and Methods**

**Sample collection**

*C. jejuni* were collected from poultry at various slaughterhouses and chicken meat from retail markets in Spain from 2010 to 2011. *C. jejuni* was identified from neck skin immediately after chilling, skinless breast meat at the end of the processing line and faecal content directly after evacuation (Ugarte-Ruiz et al., 2012). In addition, *C. jejuni* was also isolated from urban effluents at a sewage treatment plant from 2010 to 2014. For this study, 63 *C. jejuni* strains were investigated from the food chain which included 23 neck skin, 19 meat and 17 faecal isolates plus 4 isolates from urban effluents. Isolation and detection of *C. jejuni* was performed in Spain as described by Ugarte-Ruiz et al. (Ugarte-Ruiz et al., 2012). Genomic DNA (gDNA) was isolated using PureLink® Genomic DNA Mini - Life Technologies (Grand Island, NY, USA).

**Genome sequencing, assembly and annotation**

Genome sequencing of all *C. jejuni* strains was performed using Illumina MiSeq 2 × 151 bp paired-end sequencing. Initial data quality was assessed in FastQC (Andrews, 2010). The sequencing reads were quality controlled using Trimmomatic (v0.32) (‘leading’ and ‘trailing’ setting of 3, a ‘slidingwindow’ setting of 4 : 20 and a ‘minlength’ of 36 nucleotides) (Bolger et al., 2014). Reads were mapped using BWA-MEM (v0.7.7-r441) against the genome sequence of T6SS-positive *C. jejuni* 414 (CM000855) (Li and Durbin, 2009). Assembly was performed using VelvetOptimiser (v2.2.5) using n50 optimization (Zerbino and Birney, 2008; Gladman and Seemann, 2012). Contigs were ordered against *C. jejuni* 414 using ABACAS (v1.3.1) (Assfæ et al., 2009). Annotation of genomes was performed with RATT (Otto et al., 2011) using *C. jejuni* NCTC 11168 (AL11168), *C. jejuni* 414 (CM000855), *C. jejuni* RM1221 (CP000025), *C. coli* 76339 (HG326877), *C. coli* CVM N29710 (CP000466), *C. concisus* 13826 (CP000792), *C. fetus* 82-40 (CP000487), *C. jejuni* 81-176 (CP000538), *C. jejuni* M1 (CP001900) and *C. lari* RM2100 (CP000932). Genomes were visualised using Artemis and act software (Carver et al., 2012). T6SS ORFs were identified using BLAST (Altschul et al., 1990; Gish and States, 1993).

**Results**

Using T6SS nucleotide and protein sequences from *C. jejuni* strain 108 (JX436460), the genomes of the 63 Spanish isolates were analysed to identify the presence of T6SS ORFs (Table 1). Our study identified 9 of 63 (14%) isolates harbouring all 13 T6SS ORFs. These strains were from faecal, neck skin and breast meat, whereas none of the isolates from urban effluents contained any T6SS ORFs (Table 1). A total of 51 of 63 (81%) strains did not include any T6SS ORFs and were considered as negative. Three isolates named as ZTA10/00846CPD PRESTON, ZTA10/02285CPF and ZTA10/02286CPF PRESTON (representing 5% of total sample number) did not contain the whole 13 T6SS repertoire, lacking 1, 5 and 10 ORFs respectively.

In addition to using the *C. jejuni* strain 108 T6SS ORF sequences to identify T6SS in the 63 Spanish isolates, we also used the T6SS from *C. jejuni* strain 414 (CM000855). The same T6SS ORFs were identified in the 63 Spanish isolates when using T6SS nucleotide and protein sequences from *C. jejuni* strain 414. The *C. jejuni* strain 414 genome was not annotated with a T6SS and so we used the *C. jejuni* strain 108 T6SS nucleotide and protein sequences to determined the location of the *C. jejuni* strain 414 T6SS ORFs (C414_000040085 (tssD), C414_000040087 (tssM), C414_000040089 (tssF), C414_000040090 (tssL), C414_000040091 (tssK), C414_000040092 (tssJ), C414_000040093 (tssA), C414_000040095 (tssB), C414_000040096 (tssC), C414_000040097 (tssE), C414_000040098 (tssP), C414_000040099 (tssG), C414_000040100 (tssI).

**Discussion**

In this study, we found that the proportion of Spanish *C. jejuni* isolates containing all 13 T6SS ORFs was 14%, which is higher than data from previous studies that predominantly analysed strains from Europe and USA (Bleumink-Pluym et al., 2013; Harrison et al., 2014), but significantly below the rates in Vietnam (Harrison et al., 2014); noting that different sources and method for collection of samples have existed within the studies.
Table 1. T6SS from *C. jejuni* strain 108 with the respective amino acid size and matches identified in the Spanish isolates. Negative results are not shown.

<table>
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<th>Sample source</th>
<th>ZTA10/00476CPD</th>
<th>ZTA10/00846CPD</th>
<th>ZTA10/00847CPF</th>
<th>PRESTON ZTA10/01736CPF</th>
<th>ZTA10/01876CPD</th>
<th>ZTA10/01877CPD</th>
<th>ZTA10/02003CPD</th>
<th>ZTA10/02285CPF</th>
<th>ZTA10/02286CPF</th>
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<td>413 (99.5%)</td>
<td>400 (96.4%)</td>
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<td>TssC</td>
<td>481 (99.4%)</td>
<td>310 (64.0%)</td>
<td>413 (99.5%)</td>
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<td>482 (99.6%)</td>
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