SHORT COMMUNICATION

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

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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.

Keywords:
Campylobacter; T6SS; virulence; Spain

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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; Lertpiriyapong 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 (Lertpiriyapong 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 evisceration (Ugarte-Ruiz et al., 2012). In addition, *C. jejuni* was also isolated from urban effluents at a sewage water 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) (Assefa et al., 2009). Annotation of genomes was performed with RATT (Otto et al., 2011) using *C. jejuni* NCTC 11168 (AL111168), *C. jejuni* 414 (CM000855), *C. jejuni* RM1221 (CP000025), *C. coli* 76339 (HG326877), *C. coli* CVM N29710 (CP004066), *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 (*tssH*), C414_000040090 (*tssL*), C414_000040091 (*tssK*), C414_000040092 (*tssJ*), C414_000040093 (*tssA*), C414_000040095 (*tssB*), C414_000040096 (*tssC*), C414_000040097 (*tssE*), C414_000040098 (*tssF*), 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

	TssA	TssB	TssC	TssD	TssE	TssF	TssG	TssH	TssI	TssJ	TssK	TssL	TssM	
Amino acids	415	161	484	171	130	573	302	299	838	148	465	257	1175	
Reference	TssA	TssB	TssC	TssD	TssE	TssF	TssG	TssH	TssI	TssJ	TssK	TssL	TssM	Sample source
ZTA10/00476CPD	413 (99.5%)	160 (99.4%)	481 (99.4%)	171 (100%)	126 (96.9%)	567 (99.0%)	302 (100%)	294 (98.3%)	821 (98.0%)	147 (99.3%)	464 (99.8%)	257 (100%)	1169 (99.5%)	Faeces
ZTA10/00846CPD PRESTON	400 (96.4%)	54 (33.5%)	310 (64.0%)	149 (87.1%)		268 (46.8%)	99 (32.8%)	297 (99.3%)	821 (98.0%)	147 (99.3%)	278 (59.8%)	252 (98.1%)	831 (70.7%)	Meat
ZTA10/00847*CPF PRESTON	413 (99.5%)	160 (99.4%)	482 (99.6%)	141 (82.5%)	127 (97.7%)	568 (99.1%)	301 (99.7%)	298 (99.7%)	829 (98.9%)	147 (99.3%)	465 (100%)	254 (98.8%)	1111 (94.6%)	Meat
ZTA10/01736CPF PEQ	412 (99.3%)	159 (98.8%)	482 (99.6%)	171 (100%)	127 (97.7%)	568 (99.1%)	302 (100%)	297 (99.3%)	832 (99.3%)	146 (98.6%)	465 (100%)	257 (100%)	1169 (99.5%)	Faeces
ZTA10/01876CPD	413 (99.5%)	160 (99.4%)	482 (99.6%)	171 (100%)	127 (97.7%)	569 (99.3%)	302 (100%)	297 (99.3%)	820 (97.9%)	147 (99.3%)	465 (100%)	254 (98.8%)	1172 (99.7%)	Faeces
ZTA10/01877CPD	413 (99.5%)	160 (99.4%)	482 (99.6%)	171 (100%)	127 (97.7%)	569 (99.3%)	302 (100%)	297 (99.3%)	820 (97.9%)	147 (99.3%)	465 (100%)	254 (98.8%)	1172 (99.7%)	Neck skin
ZTA10/01877CPFB	413 (99.5%)	160 (99.4%)	482 (99.6%)	171 (100%)	127 (97.7%)	568 (99.1%)	301 (99.3%)	298 (99.7%)	820 (97.9%)	147 (99.3%)	465 (100%)	254 (98.8%)	1172 (99.7%)	Neck skin
ZTA10/02003CPFA	412 (99.3%)	160 (99.4%)	480 (99.6%)	171 (100%)	127 (97.7%)	569 (99.3%)	302 (100%)	294 (98.3%)	818 (97.6%)	147 (99.3%)	465 (100%)	254 (98.8%)	1168 (99.4%)	Faeces
ZTA10/02285CPF		160 (99.4%)	482 (99.6%)	171 (100%)	127 (97.7%)	223 (38.9%)		159 (53.2%)	799 (95.3%)				496 (42.2%)	Neck skin
ZTA10/02286CPF PRESTON							211 (69.9%)	187 (62.5%)	827 (98.7%)					Meat
ZTA10/02655CPF	412 (99.3%)	160 (99.4%)	481 (99.4%)	171 (100%)	126 (96.9%)	569 (99.3%)	301 (99.3%)	297 (99.3%)	824 (98.3%)	147 (99.3%)	464 (99.8%)	257 (100%)	1171 (99.7%)	Neck skin
ZTA11/00018CPD	413 (99.5%)	160 (99.4%)	482 (99.6%)	171 (100%)	127 (97.7%)	569 (99.3%)	302 (100%)	297 (99.3%)	819 (97.7%)	147 (99.3%)	465 (100%)	254 (98.8%)	1172 (99.7%)	Meat

The *hcp* gene (haemolysin coregulated protein; here denoted as *tssD*) has been noted as a key marker for the presence of T6SS and either forms a structural component similar to a cell puncturing device, or serves as a secreted effector protein that modulates host actin cytoskeleton rearrangement or cytokine production (Lertpiriyapong et al., 2012; Silverman et al., 2012; Zhou et al., 2012). Our analysis found that samples ZTA10/00846CPD PRESTON and ZTA10/02285CPF were missing one and five T6SS ORFs respectively; however, both contained the *hcp* gene. Furthermore, sample ZTA10/02286CPF PRESTON lacks 10 T6SS ORFs (including *hcp*). Thus, the *hcp* gene may not necessarily indicate the presence of a full T6SS loci (Harrison et al., 2014). We recommend whole-genome sequencing for investigating the presence of T6SS as isolates do not always contain the full repertoire of T6SS ORFs. Further research will be necessary to determine the prevalence and importance of T6SS-positive *C. jejuni* strains.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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