Identification of Possible Virulence Marker from Campylobacter jejuni Isolates

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A novel protein translocation system, the type-6 secretion system (T6SS), may play a role in virulence of Campylobacter jejuni. We investigated 181 C. jejuni isolates from humans, chickens, and environmental sources in Vietnam, Thailand, Pakistan, and the United Kingdom for T6SS. The marker was most prevalent in human and chicken isolates from Vietnam.

Campylobacter species are the principal bacterial cause of human foodborne enterocolitis worldwide (1). Despite the global significance of C. jejuni as a leading cause of diarrheal disease (2), the mechanisms of pathogenesis of C. jejuni are not well understood. Research on Campylobacter epidemiology has largely been conducted in high-income countries and therefore may not be representative of global patterns.

Recently, a novel class of protein translocation system was identified in gram-negative bacteria. This system, named the type-6 secretion system (T6SS), has been found to play roles in pathogen–pathogen and host–pathogen interactions and has a major effect on virulence in a range of pathogens, including Vibrio cholerae (3–6) (reviewed in 7,8). A functional T6SS was recently identified in C. jejuni (9,10) and found to have several roles in virulence, influencing cell adhesion, cytotoxicity toward erythrocytes, and colonization of mice (9,10). However, it is unknown whether T6SS changes the effects of these pathogens in human infection.

In this study, we aimed to determine whether presence of T6SS in C. jejuni is potentially a marker associated with more severe human disease. Moreover, because human infection with C. jejuni is often associated with contact with poultry, we investigated whether poultry harbor C. jejuni that possess T6SS.

The Study

To partially address bias toward study of C. jejuni strains from high-income countries and the under-representation of strains from Asia in previous studies, we previously sequenced the genomes of 12 clinical isolates of C. jejuni from Asia: 4 from Thailand, 3 from Pakistan, and 5 from Vietnam (J. Harrison, unpub. data; Figure 1). We noted that 8 (67%) of these isolates possessed a cluster of genes homologous to the recently described T6SS (Figure 1). This finding was in contrast to findings regarding previously sequenced C. jejuni genomes; only 10 (14%) of 71 previously sequenced C. jejuni strains possessed an apparently intact T6SS gene cluster (Figure 1; full listing of genomes is in online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/20/6/13-0635-Techapp1.pdf). Several other strains from our study and previously sequenced strains contained ≥1 T6SS genes but not a complete T6SS cluster. Figure 1 shows the presence and absence of each T6SS gene in each available genome sequence (J. Harrison, unpub. data) and the previously sequenced strains. A nonrandom distribution of T6SS can be seen across the phylogenetic diversity of C. jejuni; T6SS is limited to certain clades, and degeneration of the T6SS gene cluster apparently occurs in parallel within several of those clades (Figure 1).

Our genome sequencing analysis indicated that strains possessing a complete T6SS cluster could be distinguished by the presence of the hcp gene (Figure 1) (9,10). Therefore, we used hcp as a proxy for determining the presence of a functional T6SS in 181 C. jejuni isolates from chickens, humans, and environmental sources (collections of the Oxford University Clinical Research Unit; online Technical Appendix Table 2). We designed and used a multiplex PCR (online Technical Appendix Table 3) to screen for the presence of hcp in these isolates; the conserved C. jejuni housekeeping gene, gltA, was used as a positive control.

Of the 181 isolates, 28 originated from chickens in the United Kingdom and 21 from chickens in Vietnam. The hcp gene was found significantly more often in isolates...
Figure 1. Distribution of the type-six secretion system (T6SS) marker across the phylogenetic diversity of Campylobacter jejuni strains, as determined by multilocus sequence analysis. We generated a maximum-likelihood tree from concatenated nucleotide alignments of 31 housekeeping genes; nucleotide sequences were aligned by using MUSCLE (www.drive5.com/muscle) and masked by using GBLOCKS (http://molevol.cmima.csic.es/castresana/Gblocks.html). Maximum-likelihood analysis was done by using the GTR model in PhyML (http://code.google.com/p/phyml/). Numbers on nodes denote bootstrap values (1,000 bootstrap replicates); values <50 are not shown. Black circles indicate strains whose genomes were sequenced in this study (GenBank accession nos. AUUQ00000000, AUUP00000000, AUUO00000000, AUUN00000000, AUUM00000000, AVUL00000000, AUUK00000000, AUUJ00000000, AUUI00000000, ARWS00000000, AUUH00000000, AUUG00000000). We inferred the presence/absence of each of the T6SS genes on the basis of TBLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch) searches against the predicted proteins sequences from C. jejuni strain 414 (National Center for Biotechnology Information reference sequence no. NZ_CM000855). Presence or absence of each gene is indicated by a black or white square, respectively, for each strain: column 1, hcp; column 2, icmF_1; column 3, icmF_2; column 4, vasK; column 5, FHA; column 6, vgrg; column 7, vasE; column 8, vasD; column 9, impA; column 10, impD; column 11, impC; columns 12 and 13, conserved hypotheticals; column 14, vasA; column 15, vasB; column 16, vgrg. The sequence type (ST) and ST complex (STC) columns represent global multilocus sequence types as described by the Oxford multilocus sequence typing scheme (http://pubmlst.org). ST: unknown ST; –, isolate could not be allocated to a specific ST or STC. Scale bar indicates nucleotide substitutions per site. Further details of the isolates are provided in online Technical Appendix Table 2 (wwwnc.cdc.gov/EID/article/20/6/13-0635-Techapp1.pdf).
from Vietnam (15 [71.4%] isolates) than in those from the United Kingdom (1 [3.5%] isolate) (p<0.01 by 2-sample Z-test; online Technical Appendix Figure 1). An additional 38 of the isolates were from humans in the United Kingdom and 33 from humans in Vietnam; again, the hcp gene was significantly more prevalent in isolates from Vietnam (20 [60.6%] isolates) than those from the United Kingdom (1 [2.6%] isolate) (p<0.01 by 2-sample Z-test; online Technical Appendix Figure 2).

We also found that patients infected with hcp-positive C. jejuni experienced bloody diarrhea more commonly than those infected with hcp-negative C. jejuni. For the 36 isolates for which detailed clinical data on patients were available, 6 (31.6%) of 19 patients in Vietnam who were infected with hcp-positive C. jejuni had bloody diarrhea, compared with 1 (5.9%) of 17 patients infected with hcp-negative C. jejuni (p<0.05 by 2-sample Z-test) (Figure 2). These results suggest a potential correlation between T6SS and bloody diarrhea, a serious clinical manifestation of the infection that results in higher rates of hospitalization and greater need for treatment with antimicrobial drugs (11). Moreover, Campylobacter-related septicemia developed in the 1 patient in the United Kingdom who was infected with a T6SS-positive strain (11). These data suggest that infection with the C. jejuni T6SS genotypic strains is associated with more severe disease. However, for sample bias to be ruled out, a comprehensive study is required in which the prevalence of T6SS is measured in C. jejuni samples from patients with mild and severe forms of infection.

We found a number of C. jejuni strains from humans and poultry that possessed the T6SS cluster, although some strains showed a slightly modified gene order (online Technical Appendix Table 1 and Figure 3). However, most (61 [85.9%] of 71) of the previously sequenced C. jejuni isolates lacked a complete T6SS gene cluster (Figure 1); this finding might explain why T6SS was not discovered in C. jejuni sooner. Conversely, our PCR-based study frequently identified the hcp marker in isolates from Thailand, Pakistan, and Vietnam (Table). We cannot be certain that all of the isolates with the hcp marker possessed a complete and functional T6SS gene cluster, but the hcp gene is consistently associated with the presence of a complete T6SS cluster in all available sequenced C. jejuni genomes (Figure 1). This correlation lends confidence to the use of hcp as a proxy.

Poultry are a well-documented reservoir of human Campylobacter infection (12). We found that Campylobacter strains harboring the hcp marker were significantly associated with chickens in Asia. Large numbers of poultry are imported into North America and Europe from low-income countries, including Thailand (13). This process could introduce T6SS-positive Campylobacter genotypes into the food chains of the importing countries, posing a potential emerging threat to public health.

Conclusions

Our results suggest that the T6SS may be more prevalent in C. jejuni in Vietnam, Pakistan, and Thailand than in the United Kingdom. Furthermore, our results suggest that hcp may be a marker associated with severe human disease caused by C. jejuni infection in Vietnam, although there is no evidence that the association is causal. Chickens imported from these countries could be a source of hcp-positive strains and may have the potential to cause severe human infection.

Table. Overview of Campylobacter jejuni strains containing type-six secretion system genetic marker hcp, by country and isolate source

| Isolate source | United Kingdom | Vietnam | Pakistan | Thailand | Total |
|----------------|----------------|---------|----------|----------|-------|
| Human          | 1/38 (2.6)     | 20/33 (60.6) | 2/13 (15.4) | 1/3 (33.3) | 24/87 (27.6) |
| Chicken        | 1/28 (3.9)     | 15/21 (71.4) | 1/2 (50)   | 0        | 17/51 (33.3) |
| Other          | 5/26 (19.2)    | 1/14 (7.1)   | 1/3 (33.3) | 0        | 7/43 (16.3)  |
| Total          | 7/92 (7.6)     | 36/68 (54.4) | 4/18 (22.2) | 1/3 (33.3) | 48/181 (26.5) |

Figure 2. Percentage of hcp-positive Campylobacter jejuni strains isolated from patients in Vietnam who had bloody diarrhea and nonbloody diarrhea. Patients who were hospitalized because of C. jejuni infection were scored for the presence of bloody diarrhea or nonbloody diarrhea, and presence of the hcp type-six secretion system (T6SS) marker in strains isolated from the patients was determined. Of patients with bloody diarrhea, 32% were infected with hcp-positive strains; of patients with nonbloody diarrhea, 5% were infected with hcp-positive strains.
Acknowledgments

We thank Konrad Paszkiewicz and Karen Moore for assistance with whole-genome sequencing.

The work was partly supported by the UK Biotechnology and Biological Sciences Research Council, award BB/1024631/1 to R.T., D.S., and O.C.; by a Wellcome Trust Institutional Strategic Support Award (WT097835MF); and by a studentship awarded to J.H.

Mr Harrison is a PhD student at the University of Exeter under the supervision of D.S. His research focuses on using bioinformatic methods to investigate the comparative genomics of emerging diseases and plant-associated microbes.

References

1. Adak GK, Meakins SM, Yip H, Lopman BA, O’Brien SJ. Disease risks from foods, England and Wales, 1996–2000. Emerg Infect Dis. 2005;11:365–72. http://dx.doi.org/10.3201/eid1103.040191
2. Allos BM. Campylobacter jejuni infections: update on emerging issues and trends. Clin Infect Dis. 2001;32:1201–6. http://dx.doi.org/10.1086/319760
3. Das S, Chakrabortty A, Banerjee R, Roychoudhury S, Chaudhuri K. Comparison of global transcription responses allows identification of Vibrio cholerae genes differentially expressed following infection. FEMS Microbiol Lett. 2000;190:87–91. http://dx.doi.org/10.1111/j.1574-6968.2000.tb09267.x
4. Ishikawa T, Sabharwal D, Bröms J, Milton DL, Sjöstedt A, Uhlin BE, et al. Pathoadaptive conditional regulation of the type VI secretion system in Vibrio cholerae O1 strains. Infect Immun. 2012;80:575–84. http://dx.doi.org/10.1128/IAI.00510-11
5. Parsons DA, Heffron F. sciS, an icmF homolog in Salmonella enterica serovar Typhimurium, limits intracellular replication and decreases virulence. Infect Immun. 2005;73:4338–45. http://dx.doi.org/10.1128/IAI.73.7.4338-4345.2005
6. Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, et al. Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. Proc Natl Acad Sci U S A. 2006;103:1528–33. http://dx.doi.org/10.1073/pnas.0510322103
7. Cascales E. The type VI secretion toolkit. EMBO Rep. 2008;9:735–41. http://dx.doi.org/10.1038/embor.2008.131
8. Mulder DT, Cooper CA, Coombes BK. Type VI secretion system-associated gene clusters contribute to pathogenesis of Salmonella enterica serovar Typhimurium. Infect Immun. 2012;80:1996–2007. http://dx.doi.org/10.1128/IAI.016205-11
9. Lertpiriyapong K, Gamazon ER, Feng Y, Park DS, Pang J, Botka G, et al. Campylobacter jejuni type VI secretion system: roles in adaptation to deoxycholic acid, host cell adherence, invasion, and in vivo colonization. PLoS ONE. 2012;7:e42842. http://dx.doi.org/10.1371/journal.pone.0042842
10. Bleumink-Pluym NMC, van Alphen LB, Bouwman LI, Wösten MMSM, van Putten JPM. Identification of a functional type VI secretion system in Campylobacter jejuni conferring capsule polysaccharide sensitive cytotoxicity. PLoS Pathog. 2013;9:e1003393. http://dx.doi.org/10.1371/journal.ppat.1003393
11. Kuskonmaz B, Yurdaköök K, Yalçın SS, Ozment E. Comparison of acute bloody and watery diarrhea: a case control study. Turk J Pediatr. 2009;51:133–40.
12. Harris NV, Weiss NS, Nolan CM. The role of poultry and meats in the etiology of Campylobacter jejuni/coli enteritis. Am J Public Health. 1986;76:407–11. http://dx.doi.org/10.2105/AJPH.76.4.407
13. Food and Agriculture Organization of the United Nations. Agribusiness handbook: poultry meat and eggs. 2010 [cited 2013 Apr 1]. http://www.fao.org/fileadmin/user_upload/tci/docs/1_AH9-Poultry%20Meat%20&%20Eggs.pdf

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Technical Appendix

Supplementary Methods

Multiplex PCR analysis was used for identification of the hcp and gltA genes. Oligonucleotide primers were designed using the Cj1 sequence (Technical Appendix Table 3). PCRs were carried out in a volume of 25 μl. This reaction consisted of 0.2 mM of each dNTP (Invitrogen), 0.25 unit of TaqDNA polymerase (BioLabs), 0.4 μM of the downstream and upstream hcp primer, and 1–100 ng of template DNA. PCR was carried out with a DNA Engine Peltier Thermal Cycler (Bio Rad). Primers for internal control gene gltA were added at a concentration of 0.05 μM in each of the reaction mixtures to check the fidelity of the PCR. A reaction mixture without template DNA was used as the negative control. DNA extracted from C. jejuni clinical isolate Cj1 was used a positive control for amplification of primers. PCR products were analyzed on 1.5% agarose gel stained with SYBR Safe DNA stain.

Technical Appendix Table 1. List of C. jejuni strains included in MLSA analysis.

| strain name | source  | country of origin | T6SS  | Genome status | Ref | Hcp +ve |
|-------------|---------|-------------------|-------|---------------|-----|--------|
| 305         | Turkey  | Germany           | negative | draft |       |        |
| 327         | Turkey  | Unknown           | negative | draft |       |        |
| 414         | Bank Vole | Unknown       | positive | complete | [3] | yes    |
| 1213        | Cow     | USA               | negative | draft |       |        |
| 1336        | Bird    | Unknown           | positive | complete | [3] |        |
| 1577        | Cow     | USA               | negative | draft |       |        |
| 1798        | Cow     | USA               | negative | draft |       |        |
| 1854        | Cow     | USA               | negative | draft |       |        |
| 1893        | Cow     | USA               | negative | draft |       |        |
| 1928        | Cow     | USA               | negative | draft |       |        |
| 04197       | Unknown | Unknown           | negative | draft |       |        |
| 04199       | Unknown | Unknown           | negative | draft |       |        |
| 6399        | Unknown | Unknown           | negative | draft |       |        |
| 51037       | chicken | USA               | positive | draft | yes   |        |
| 51494       | chicken | USA               | positive | draft | yes   |        |
| 53161       | chicken | USA               | positive | draft |       |        |
| 60004       | chicken | USA               | negative | draft |       |        |
| 81116       | human   | Unknown           | negative | complete | [4] |        |
| 86605       | chicken | USA               | negative | draft |       |        |
| 87330       | chicken | USA               | negative | draft |       |        |
| 87459       | chicken | USA               | positive | draft |       |        |
| 110_21      | Unknown | USA               | negative | draft |       |        |
| 129_258     | Cow     | USA               | negative | draft |       |        |
| 140_16      | Cow     | USA               | negative | draft |       |        |
| strain name | source     | country of origin | T6SS   | Genome status | Ref   | Hcp +ve |
|------------|------------|-------------------|--------|---------------|-------|---------|
| 1997_1     | Human      | USA               | negative | draft         |       |         |
| 1997_10    | Human      | USA               | positive | draft         |       | yes     |
| 1997_11    | Human      | USA               | negative | draft         |       |         |
| 1997_14    | Human      | USA               | positive | draft         |       | yes     |
| 1997_4     | Human      | USA               | negative | draft         |       |         |
| 1997_7     | Human      | USA               | negative | draft         |       |         |
| 2008_1025  | Human      | France            | negative | draft         |       |         |
| 2008_831   | Human      | France            | negative | draft         |       |         |
| 2008_872   | Human      | France            | negative | draft         |       |         |
| 2008_894   | Human      | France            | negative | draft         |       |         |
| 2008_979   | Human      | France            | positive | draft         |       | yes     |
| 2008_988   | Human      | France            | negative | draft         |       |         |
| 260_94     | Human      | S. Africa         | negative | draft         |       |         |
| 81_176     | Human      | Unknown           | negative | Complete      | [5]   |         |
| 84_25      | Human      | Unknown           | negative | Complete      | [6]   |         |
| ATCC_33560 | Cow        | Brussels          | positive | draft         |       | yes     |
| CG8421     | Human      | Thailand          | negative | draft         | [7]   |         |
| CG8486     | Human      | Thailand          | negative | draft         | [8]   |         |
| D2600      | Human      | USA               | negative | draft         | [9]   |         |
| DFVF1099   | chicken    | Unknown           | negative | draft         | [10]  |         |
| H22082     | Human      | New Zealand       | negative | draft         | [11]  |         |
| HB93_13    | Human      | China             | negative | draft         | [12]  |         |
| IA3902     | Sheep      | USA               | negative | Complete      | [13]  |         |
| TCDCCJ07001| Human      | Unknown           | negative | draft         | [14]  |         |
| LMG_23210  | chicken    | Belgium           | positive | draft         | [15]  |         |
| LMG_23211  | chicken    | Belgium           | negative | draft         | [16]  |         |
| LMG_23216  | chicken    | Belgium           | positive | draft         |       |         |
| LMG_23218  | chicken    | Belgium           | negative | draft         |       |         |
| LMG_23223  | chicken    | Belgium           | positive | draft         |       |         |
| LMG_23263  | chicken    | Bosnia and Herzegovina | positive | draft     | [17]  |         |
| LMG_23264  | Human      | Slovenia          | negative | draft         |       |         |
| LMG_23269  | chicken    | Belgium           | negative | draft         |       |         |
| LMG_23357  | water      | Netherlands       | positive | draft         |       |         |
| LMG_9081   | human      | USA               | negative | draft         |       |         |
| LMG_9217   | Human      | Belgium           | negative | draft         |       |         |
| LMG_9872   | Human      | Sweden            | negative | draft         |       |         |
| LMG_9879   | Human      | Canada            | negative | draft         |       |         |
| M1         | Human/poultry | Unknown           | negative | complete      | [12]  |         |
| NCTC11168  | Human      | Unknown           | negative | complete      | [13]  |         |
| NW         | Human      | USA               | positive | draft         | [14]  |         |
| P110B      | chicken    | New Zealand       | negative | draft         | [15]  |         |
| P854       | chicken    | UK                | positive | draft         |       | yes     |
| RM1221     | Unknown    | Unknown           | positive | complete      | [16]  |         |
| S3         | poultry    | Unknown           | negative | complete      | [17]  |         |
| Doylei 269 97 | Human | Unknown           | negative | Complete      |       |         |
| xy259      | Unknown    | Unknown           | negative | draft         |       |         |
| 55037      | chicken    | USA               | negative | draft         |       |         |

Technical Appendix Table 2. List of 181 C. jejuni strains analyzed in this study.

| strain name | source     | country of origin | T6SS   | Genome status | Ref   | Strain source |
|------------|------------|-------------------|--------|---------------|-------|--------------|
| 28766      | Beach      | UK                | negative |              |       | This study    |
| KSCattle8  | Cattle     | UK                | negative |              |       | This study    |
| 11974      | human      | UK                | negative |              |       | This study    |
| 13305      | human      | UK                | negative |              |       | This study    |
| 11919      | human      | UK                | negative |              |       | This study    |
| 30280      | human      | UK                | negative |              |       | This study    |
| 11818      | human      | UK                | negative |              |       | This study    |
| 12241      | human      | UK                | negative |              |       | This study    |
| 99/188     | human      | UK                | negative |              |       | This study    |
| 99/197     | human      | UK                | negative |              |       | This study    |
| 99/97      | human      | UK                | negative |              |       | This study    |
| 0 1/ 43    | human      | UK                | negative |              |       | This study    |
| strain name | source | country of origin | T6SS | Genome status | Ref | Strain source |
|-------------|--------|-------------------|------|---------------|-----|---------------|
| 99/189      | human  | UK                | negative | This study    |     |               |
| 99/216      | human  | UK                | negative | This study    |     |               |
| 94/229      | human  | UK                | negative | This study    |     |               |
| 99/12       | human  | UK                | negative | This study    |     |               |
| BB1267      | human  | UK                | negative | This study    |     |               |
| 31467       | human  | UK                | negative | This study    |     |               |
| 31484       | human  | UK                | negative | This study    |     |               |
| 32799       | human  | UK                | negative | This study    |     |               |
| 31485       | human  | UK                | negative | This study    |     |               |
| 33084       | human  | UK                | positive | This study    |     |               |
| 93/372      | human  | UK                | negative | This study    |     |               |
| 32787       | human  | UK                | negative | This study    |     |               |
| 44119       | human  | UK                | negative | This study    |     |               |
| 47693       | human  | UK                | negative | This study    |     |               |
| 33106       | human  | UK                | negative | This study    |     |               |
| 34007       | human  | UK                | negative | This study    |     |               |
| Hi4080306   | human  | UK                | negative | This study    |     |               |
| 90843       | human  | UK                | negative | This study    |     |               |
| Hi4050471   | human  | UK                | negative | This study    |     |               |
| Hi40620306  | human  | UK                | negative | This study    |     |               |
| BB1267      | human  | UK                | negative | This study    |     |               |
| Hi81266     | human  | UK                | negative | This study    |     |               |
| Hi80586     | human  | UK                | negative | This study    |     |               |
| Hi80547     | human  | UK                | negative | This study    |     |               |
| Hi81006     | human  | UK                | negative | This study    |     |               |
| KSSAPSM6    | human  | UK                | negative | This study    |     |               |
| Hi81214     | human  | UK                | negative | This study    |     |               |
| KSSHPSM4    | human  | UK                | negative | This study    |     |               |
| 99/118      | Cow    | UK                | negative | This study    |     |               |
| 99/201      | Cow    | UK                | negative | This study    |     |               |
| 99/202      | Cow    | UK                | negative | This study    |     |               |
| C0599 3095  | Cow    | UK                | negative | This study    |     |               |
| C085 40995  | Cow    | UK                | negative | This study    |     |               |
| 1182 ENV    | Env    | UK                | negative | This study    |     |               |
| PS304       | Pig    | UK                | negative | This study    |     |               |
| PS623       | Pig    | UK                | positive  | This study    |     |               |
| PS762       | Pig    | UK                | negative | This study    |     |               |
| PS830       | Pig    | UK                | negative | This study    |     |               |
| PS838       | Pig    | UK                | negative | This study    |     |               |
| PS843       | Pig    | UK                | positive  | This study    |     |               |
| PS849       | Pig    | UK                | positive  | This study    |     |               |
| PS852       | Pig    | UK                | positive  | This study    |     |               |
| PS857       | Pig    | UK                | positive  | This study    |     |               |
| C120/2      | Poultry | UK              | negative | This study    |     |               |
| C132/1      | Poultry | UK              | negative | This study    |     |               |
| D2/T/80     | Poultry | UK              | negative | This study    |     |               |
| PS55491     | Poultry | UK              | positive  | This study    |     |               |
| A83515A     | Poultry | UK              | negative | This study    |     |               |
| A1CF12      | Poultry | UK              | negative | This study    |     |               |
| D502009A    | Poultry | UK              | negative | This study    |     |               |
| C3/T2/8     | Poultry | UK              | negative | This study    |     |               |
| D2/27B      | Poultry | UK              | negative | This study    |     |               |
| C3/T2/25    | Poultry | UK              | negative | This study    |     |               |
| EX1286      | Poultry | UK              | negative | This study    |     |               |
| MB1         | Poultry | UK              | negative | This study    |     |               |
| MB2         | Poultry | UK              | negative | This study    |     |               |
| MB3         | Poultry | UK              | negative | This study    |     |               |
| MB4         | Poultry | UK              | negative | This study    |     |               |
| MB5         | Poultry | UK              | negative | This study    |     |               |
| MB6         | Poultry | UK              | negative | This study    |     |               |
| MB7         | Poultry | UK              | negative | This study    |     |               |
| MB8         | Poultry | UK              | negative | This study    |     |               |
| MB9         | Poultry | UK              | negative | This study    |     |               |
| MB10        | Poultry | UK              | negative | This study    |     |               |
| strain name | source | country of origin | T6SS | Genome status | Ref | Strain source |
|-------------|--------|-------------------|------|---------------|-----|---------------|
| MB12        | Poultry | UK                | negative | This study |
| MB13        | Poultry | UK                | negative | This study |
| MB14        | Poultry | UK                | negative | This study |
| MB15        | Poultry | UK                | negative | This study |
| MB16        | Poultry | UK                | negative | This study |
| MB17        | Poultry | UK                | negative | This study |
| MB18        | Poultry | UK                | negative | This study |
| S2160509901 | Sheep  | UK                | negative | This study |
| S3902099903 | Sheep  | UK                | negative | This study |
| S1200409904 | Sheep  | UK                | negative | This study |
| S8704099    | Sheep  | UK                | negative | This study |
| S3720509904 | Sheep  | UK                | negative | This study |
| S3790809903 | Sheep  | UK                | negative | This study |
| S43503099   | Sheep  | UK                | negative | This study |
| S4990109905 | Sheep  | UK                | negative | This study |
| S58503099   | Sheep  | UK                | negative | This study |
| Cj 54       | Camel  | Pakistan          | negative | This study |
| AKRH011     | human  | Pakistan          | negative | This study |
| 702         | human  | Pakistan          | negative | This study |
| Y25         | human  | Pakistan          | negative | This study |
| 2960HF      | human  | Pakistan          | negative | This study |
| 712         | human  | Pakistan          | negative | This study |
| K1          | human  | Pakistan          | negative | draft |
| K2          | human  | Pakistan          | positive  | This study |
| K4          | human  | Pakistan          | negative | This study |
| K5          | human  | Pakistan          | negative | draft |
| K6          | human  | Pakistan          | negative | This study |
| K7          | human  | Pakistan          | negative | This study |
| K8          | human  | Pakistan          | positive  | This study |
| 80          | Poultry | Pakistan          | positive  | draft |
| Cj245       | waste water | Pakistan    | negative | This study |
| Cj 236      | waste water | Pakistan    | positive  | This study |
| Cj1         | human  | Thailand          | positive  | draft |
| Cj2         | human  | Thailand          | negative  | draft |
| Cj3         | human  | Thailand          | negative  | draft |
| Cj5         | human  | Thailand          | positive  | draft |
| 20157       | human  | Vietnam           | positive  | This study |
| 30286       | human  | Vietnam           | positive  | draft |
| 30261       | human  | Vietnam           | positive  | This study |
| 10227       | human  | Vietnam           | positive  | draft |
| 20160       | human  | Vietnam           | negative  | This study |
| 30106       | human  | Vietnam           | negative  | This study |
| 20288       | human  | Vietnam           | negative  | This study |
| 30311       | human  | Vietnam           | positive  | This study |
| 20283       | human  | Vietnam           | positive  | This study |
| 10186       | human  | Vietnam           | positive  | draft |
| 20176       | human  | Vietnam           | positive  | draft |
| 20231       | human  | Vietnam           | positive  | This study |
| 20301       | human  | Vietnam           | positive  | This study |
| 30318       | human  | Vietnam           | positive  | draft |
| 20321       | human  | Vietnam           | positive  | This study |
| 20332       | human  | Vietnam           | negative  | This study |
| 30355       | human  | Vietnam           | positive  | This study |
| 20319       | human  | Vietnam           | positive  | This study |
| 20137       | human  | Vietnam           | positive  | This study |
| 30391       | human  | Vietnam           | negative  | This study |
| 30396       | human  | Vietnam           | negative  | This study |
| T0275       | human  | Vietnam           | negative  | This study |
| 20227       | human  | Vietnam           | positive  | This study |
| 30446       | human  | Vietnam           | positive  | This study |
| 20127       | human  | Vietnam           | positive  | This study |
| Strain name | Source | Country of origin | T6SS | Genome status | Ref | Strain source |
|------------|--------|-------------------|------|---------------|-----|--------------|
| 20396      | human  | Vietnam           |      | negative      |     | This study    |
| 10126      | human  | Vietnam           |      | positive      |     | This study    |
| 20084      | human  | Vietnam           |      | negative      |     | This study    |
| 30431      | human  | Vietnam           |      | negative      |     | This study    |
| 30146      | human  | Vietnam           |      | negative      |     | This study    |
| 10070      | human  | Vietnam           |      | negative      |     | This study    |
| 10152      | human  | Vietnam           |      | negative      |     | This study    |
| 20245      | human  | Vietnam           |      | positive      |     | This study    |
| 71V103     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V42      | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V489     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V151     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V135     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V445     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V484     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V420     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V409     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V137     | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V49      | Duck   | Vietnam           |      | negative      |     | This study    |
| 71V69      | Duck   | Vietnam           |      | negative      |     | This study    |
| 72H57      | Pig    | Vietnam           |      | negative      |     | This study    |
| 71V110     | Duck   | Vietnam           |      | positive      |     | This study    |
| 71G139     | Chicken| Vietnam           |      | negative      |     | This study    |
| 71G142     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G356     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G570     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G784     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G998     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G1212    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G1426    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G1640    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G1854    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G2068    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G2282    | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G326     | Chicken| Vietnam           |      | negative      |     | This study    |
| 71G143     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G143     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G125     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G124     | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G90      | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G30      | Chicken| Vietnam           |      | positive      |     | This study    |
| 71G43      | Chicken| Vietnam           |      | negative      |     | This study    |
| 72G117     | Chicken| Vietnam           |      | negative      |     | This study    |

*Boldface indicates strains used in both the MLSA analysis and molecular epidemiology.

Technical Appendix Table 3. Primers used to PCR amplify the hcp and gltA genes

| Primers (for target genes) | Primer sequence (5’→3’) | Predicted amplicon size | Tm | Reference |
|---------------------------|-------------------------|-------------------------|----|-----------|
| gltA F C                  | GCCCAAGGCCATCAAGCGGA     | 142 bp                  | 60 | This study |
| gltA F C                  | GCGCTTGGGCGCTGCTGCAA     |                         | 58 | This study |
| Hcp F                     | CAAGCGGTGATCTACTGAAA     | 463 bp                  | 60 | This study |
| Hcp R                     | TAAGCTTTGCCCCTCTCTCCA    |                         | 60 | This study |
Technical Appendix Figure 1. Prevalence of T6SS genetic marker hcp in Campylobacter jejuni isolated from chickens in Vietnam and the UK. Multiplex PCR was performed on genomic DNA purified from C. jejuni that were isolated from chickens from Vietnam and the UK. Conserved T6SS gene hcp was used as a marker for a complete T6SS cluster. The conserved housekeeping gene gltA was used as a positive control.

Technical Appendix Figure 2. Prevalence of T6SS genetic marker hcp in Campylobacter jejuni isolated from humans in Vietnam and the UK. Multiplex PCR was performed on genomic DNA purified from C. jejuni that were isolated from humans from Vietnam and the UK. Conserved T6SS gene hcp was used as a marker for a complete T6SS cluster. The conserved housekeeping gene gltA was used as a positive control.
Technical Appendix Figure 3. Comparison of the gene orders in the T6SS gene clusters found in *Campylobacter jejuni*. The figure shows BLASTN alignments between representatives of each of the two gene-order types: strain 10227 shares gene order with P854, ATCC 33560, 2008 979, LMG 23263, 30318, Cj1, 1997 14, 51037, 51494, 1997 10, Cj5, LMG 23210, 10186, 255, 20176. Strain 414 has a unique gene arrangement. Strains NW and LMG23357 are examples of partial T6SS gene clusters (vgrG is shown as absent from LMG23357 but is present in the assembly on a different contig). Figure visualised using Artemis comparison tool.

References

1. Takamiya M, Ozen A, Rasmussen M, Alter T, Gilbert T, Ussery DW, et al. Genome sequences of two stress-tolerant *Campylobacter jejuni* poultry strains, 305 and DFVF1099. J Bacteriol. 2011a;193:5546–7. PubMed [http://dx.doi.org/10.1128/JB.05753-11](http://dx.doi.org/10.1128/JB.05753-11)

2. Takamiya M, Ozen A, Rasmussen M, Alter T, Gilbert T, Ussery DW, et al. Genome Sequence of *Campylobacter jejuni* strain 327, a strain isolated from a turkey slaughterhouse. Stand Genomic Sci. 2011b;4:113–22. PubMed [http://dx.doi.org/10.4056/sigs.1313504](http://dx.doi.org/10.4056/sigs.1313504)

3. Hepworth PJ, Ashelford KE, Hinds J, Gould K, Witney AA, Williams NJ, et al. Genomic variations define divergence of water/wildlife-associated *Campylobacter jejuni* niche specialists from
common clonal complexes. Environ Microbiol. 2011;13:1549–60. PubMed [http://dx.doi.org/10.1111/j.1462-2920.2011.02461.x]

4. Pearson BM, Gaskin DJH, Segers RPM, Wells JM, Nuijten PJM, van Vliet AHM. The complete genome sequence of _Campylobacter jejuni_ strain 81116 (NCTC11828). J Bacteriol. 2007;189:8402–3. PubMed [http://dx.doi.org/10.1128/JB.01404-07]

5. Russell RG, Blaser MJ, Sarmiento JI, Fox J. Experimental _Campylobacter jejuni_ infection in Macaca nemestrina. Infect Immun. 1989;57:1438–44. PubMed

6. Poly F, Read TD, Chen Y-H, Monteiro M, Serichantalergs O, Pootong P, et al. Characterization of two _Campylobacter jejuni_ strains for use in volunteer experimental-infection studies. Infect Immun. 2008;76:5655–67. PubMed [http://dx.doi.org/10.1128/IAI.00780-08]

7. Poly F, Read T, Tribble DR, Baqar S, Lorenzo M, Guerry P. Genome Sequence of a Clinical Isolate of _Campylobacter jejuni_. Infect Immun. 2007;75:3425–33. PubMed [http://dx.doi.org/10.1128/IAI.00050-07]

8. Jerome JP, Klahn BD, Bell J, Barrick JE, Brown CT, Mansfield LS. Draft Genome Sequences of Two _Campylobacter jejuni_ Clinical Isolates, NW and D2600. J Bacteriol. 2012;194:5707–8. PubMed [http://dx.doi.org/10.1128/JB.01338-12]

9. Burrough ER, Sahin O, Plummer PJ, Zhang Q, Yaeger MJ. Pathogenicity of an emergent, ovine abortifacient _Campylobacter jejuni_ clone orally inoculated into pregnant guinea pigs. Am J Vet Res. 2009;70:1269–76. PubMed [http://dx.doi.org/10.2460/ajvr.70.10.1269]

10. Luo Y, Sahin O, Dai L, Sippy R, Wu Z, Zhang Q. Development of a Loop-Mediated Isothermal Amplification Assay for Rapid, Sensitive and Specific Detection of a _Campylobacter jejuni_ Clone. J Vet Med Sci. 2012;74:591–6. PubMed [http://dx.doi.org/10.1292/jvms.11-0462]

11. Zhang M, Li Q, He L, Meng F, Gu Y, Zheng M, et al. Association Study Between an Outbreak of Guillain-Barre Syndrome in Jilin, China, and Preceding _Campylobacter jejuni_ Infection. Foodborne Pathog Dis. 2010;7:913–9. PubMed [http://dx.doi.org/10.1089/fpd.2009.0493]

12. Friis C, Wassenaar TM, Javed M, Snipen L, Lagesen K, Hallin PF, et al. Genomic characterization of _Campylobacter jejuni_ strain M1. PLoS ONE. 2010;5:e12253. PubMed [http://dx.doi.org/10.1371/journal.pone.0012253]

13. Gundogdu O, Bentley SD, Holden MT, Parkhill J, Dorrell N, Wren BW. Re-annotation and re-analysis of the _Campylobacter jejuni_ NCTC11168 genome sequence. BMC Genomics. 2007;8:162. PubMed [http://dx.doi.org/10.1186/1471-2164-8-162]
14. Biggs PJ, Fearnhead P, Hotter G, Mohan V, Collins-Emerson J, Kwan E, et al. Whole-genome comparison of two *Campylobacter jejuni* isolates of the same sequence type reveals multiple loci of different ancestral lineage. PLoS ONE. 2011;6:e27121. PubMed http://dx.doi.org/10.1371/journal.pone.0027121

15. Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko D, Ravel J, et al. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. PLoS Biol. 2005;3:e15. PubMed http://dx.doi.org/10.1371/journal.pbio.0030015

16. Cooper KK, Cooper M. a, Zuccolo, A., Law, B., & Joens, L. Complete genome sequence of *Campylobacter jejuni* strain S3. J Bacteriol. 2011;193:1491–2. PubMed http://dx.doi.org/10.1128/JB.01475-10