

SUPPLEMENTAL MATERIAL

Campylobacter coli* clade 3 isolates induce rapid cell death *in vitro

Cecilia Johansson, Anna Nilsson, René Kaden, and Hilpi Rautelin

TABLE S1. *C. coli* clade 1 (n=11), clade 2 (n=24) and clade 3 (n=56) isolates included in genomic analyses.

| Clade | Isolate | NCBI accession number | Source | Reference |
|--------------|-------------------------|----------------------------------|--------------------------------------|------------------|
| 1 | BIGS0002 | ANGM01000000 | Clinical | (1) |
| 1 | BIGS0005 | ANGP01000000 | Pig feces | (1) |
| 1 | BIGS0015 | ANGZ01000000 | Pig feces | (1) |
| 1 | BIGS0017 | ANHB01000000 | Chicken meat | (1) |
| 1 | BIGS0019 | ANHD01000000 | Clinical | (1) |
| 1 | BIGS0021 | ANHF01000000 | Chicken meat | (1) |
| 1 | F3 | QYUT01000000 | Clinical | This study, (2) |
| 1 | F4 | QYUS01000000 | Clinical | This study, (2) |
| 1 | F8 | QYUU01000000 | Clinical | This study, (2) |
| 1 | LMG 6440 (NTCT11366) | QZCI01000000 | Pig faeces Reference strain | This study |
| 1 | RM2228 | AAFL01000000 | Chicken carcass, Reference strain | (3) |
| 2 | A12B2 | FBGG01000000 | Environmental waters | |
| 2 | B15B3 | FBFU01000000 | Environmental waters | |
| 2 | BIGS0012 | ANGW01000000 | Clinical | (1) |
| 2 | F14B4C4 | FBGM01000000 | Environmental waters | |
| 2 | F15D2 | FBFW01000000 | Environmental waters | |
| 2 | H043880653 | FBNC01000000 | Clinical | |
| 2 | H054340287 | FBOJ01000000 | Clinical | |
| 2 | H055260513 | FBNY01000000 | Environmental waters | |

| | | | | |
|---|------------|--------------|----------------------|-----|
| 2 | H055260517 | FAXG01000000 | Environmental waters | |
| 2 | H091320798 | FBKV01000000 | Environmental waters | |
| 2 | H091940890 | FBOS01000000 | Environmental waters | |
| 2 | H092560131 | FBKJ01000000 | Environmental waters | |
| 2 | H093580632 | FBPP01000000 | Environmental waters | |
| 2 | H094560718 | FBKY01000000 | Environmental waters | |
| 2 | H105160209 | FBQJ01000000 | Environmental waters | |
| 2 | K11 | FBGA01000000 | Environmental waters | |
| 2 | M2D2 | FBGB01000000 | Environmental waters | |
| 2 | M4A4 | FBGH01000000 | Environmental waters | |
| 2 | M5D4 | FBQV01000000 | Environmental waters | |
| 2 | VA6 | MPIQ01000000 | Environmental waters | (4) |
| 2 | VA8 | MPIS01000000 | Environmental waters | (4) |
| 2 | VA24 | MPIU01000000 | Environmental waters | (4) |
| 2 | VA37 | MPIV01000000 | Environmental waters | (4) |
| 2 | VA46 | MPIX01000000 | Environmental waters | (4) |
| 3 | BIGS0003 | ANGN01000000 | Environmental waters | (1) |
| 3 | BIGS0009 | ANGT01000000 | Clinical | (1) |
| 3 | C9A1 | FBQZ01000000 | Environmental waters | |
| 3 | D13A4 | FBFX01000000 | Environmental waters | |
| 3 | H042280284 | FBQS01000000 | Clinical | |
| 3 | H042840283 | FBPA01000000 | Clinical | |
| 3 | H043560441 | FBOR01000000 | Clinical | |
| 3 | H051960469 | FBOD01000000 | Clinical | |
| 3 | H053120426 | FBMY01000000 | Environmental waters | |

| | | | |
|---|-------------|--------------|----------------------|
| 3 | H060740717 | FBIP01000000 | Environmental waters |
| 3 | H060960756 | FBGE01000000 | Environmental waters |
| 3 | H060960757 | FBHG01000000 | Environmental waters |
| 3 | H061680633 | FBOE01000000 | Environmental waters |
| 3 | H061980521b | FBNK01000000 | Environmental waters |
| 3 | H063720401 | FBPO01000000 | Clinical |
| 3 | H065100506 | FBKP01000000 | Environmental waters |
| 3 | H065160534 | FBJV01000000 | Environmental waters |
| 3 | H070380306 | FBKT01000000 | Clinical |
| 3 | H071960681 | FBNO01000000 | Environmental waters |
| 3 | H072620566 | FBKO01000000 | Environmental waters |
| 3 | H072760517 | FBQO01000000 | Clinical |
| 3 | H073180383 | FAZM01000000 | Clinical |
| 3 | H073220518 | FBNQ01000000 | Environmental waters |
| 3 | H073580401 | FBAL01000000 | Environmental waters |
| 3 | H074080509 | FBOA01000000 | Environmental waters |
| 3 | H074080511 | FBNP01000000 | Environmental waters |
| 3 | H075140555 | FBKA01000000 | Environmental waters |
| 3 | H075200522 | FBMZ01000000 | Environmental waters |
| 3 | H081380695a | FBNU01000000 | Environmental waters |
| 3 | H081840386 | FBLJ01000000 | Clinical |
| 3 | H082280513 | FAZO01000000 | Environmental waters |
| 3 | H082280515 | FAZW01000000 | Environmental waters |
| 3 | H082560569 | FAYV01000000 | Environmental waters |
| 3 | H083420694 | FBNG01000000 | Environmental waters |

| | | | | |
|---|-------------|--------------|----------------------|-----|
| 3 | H083420701 | FBNT01000000 | Environmental waters | |
| 3 | H085160742 | FBJZ01000000 | Environmental waters | |
| 3 | H085160749 | FBLD01000000 | Environmental waters | |
| 3 | H090980249 | FBKL01000000 | Environmental waters | |
| 3 | H091000385 | FBNE01000000 | Clinical | |
| 3 | H092260569b | FBNM01000000 | Environmental waters | |
| 3 | H093580324 | FBKN01000000 | Environmental waters | |
| 3 | H094560713 | FBKD01000000 | Environmental waters | |
| 3 | H094560720 | FBKQ01000000 | Environmental waters | |
| 3 | H094860392 | FBAT01000000 | Environmental waters | |
| 3 | H102740169 | FBPR01000000 | Environmental waters | |
| 3 | H110340458 | FBQC01000000 | Environmental waters | |
| 3 | H113760461 | FBOH01000000 | Clinical | |
| 3 | H124500577 | FBOV01000000 | Clinical | |
| 3 | H124620276a | FBOI01000000 | Clinical | |
| 3 | H132840800 | FBPJ01000000 | Environmental waters | |
| 3 | H133380250 | FBQM01000000 | Clinical | |
| 3 | K3D1 | FBFT01000000 | Environmental waters | |
| 3 | VA7 | MPIR01000000 | Environmental waters | (4) |
| 3 | VA15 | MPIT01000000 | Environmental waters | (4) |
| 3 | VA38 | MPIW01000000 | Environmental waters | (4) |
| 3 | 76339 | HG326877 | Clinical | (5) |

SUPPLEMENTAL REFERENCES

1. Sheppard SK, Didelot X, Jolley KA, Darling AE, Pascoe B, Méric G, Kelly DJ, Cody A, Colles FM, Strachan NJ, Ogden ID, Forbes K, French NP, Carter P, Miller WG, McCarthy ND, Owen R, Litrup E, Egholm M, Affourtit JP, Bentley SD, Parkhill J, Maiden MC, Falush D. 2013. Progressive genome-wide introgression in agricultural *Campylobacter coli*. *Mol Ecol* 22:1051-64.
2. Feodoroff FB, Lauhio AR, Sarna SJ, Hanninen ML, Rautelin HI. 2009. Severe diarrhoea caused by highly ciprofloxacin-susceptible *Campylobacter* isolates. *Clin Microbiol Infect* 15:188-92.
3. Fouts DE, Mongodin EF, Mandrell RE, Miller WG, Rasko DA, Ravel J, Brinkac LM, DeBoy RT, Parker CT, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Sullivan SA, Shetty JU, Ayodeji MA, Shvartsbeyn A, Schatz MC, Badger JH, Fraser CM, Nelson KE. 2005. Major structural differences and novel potential virulence mechanisms from the genomes of multiple *campylobacter* species. *PLoS Biol* 3:e15.
4. Nilsson A, Skarp A, Johansson C, Kaden R, Engstrand L, Rautelin H. 2018. Characterization of Swedish *Campylobacter coli* clade 2 and clade 3 water isolates. *Microbiologyopen* doi:10.1002/mbo3.583.
5. Skarp-de Haan CPA, Culebro A, Schott T, Revez J, Schweda EK, Hänninen ML, Rossi M. 2014. Comparative genomics of unintrogressed *Campylobacter coli* clades 2 and 3. *BMC Genomics* 15:129.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Neighbour-joining tree based on protein sequence alignments of CadF from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Node colours indicate clades; Clade 1 – blue, Clade 2 – green and Clade 3 – red. The exact positions of the isolates in this study are shown. Scale bar represent 0.01.

Figure S2. Alignment of CadF protein sequences from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Dash; amino acid is missing, grey box; site with variation, asterisk; site where clade 3 isolates differ from clade 1 and 2 isolates, bold; site where clade 3 is identical to *C. jejuni*, box; conserved fibronectin binding site, underlined sequence; OmpA-like domain (amino acids 167-324).

Figure S3. Neighbour-joining tree based on protein sequence alignments of CeuE from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Node colours indicate clades; Clade 1 – blue, Clade 2 – green and Clade 3 – red. The exact positions of the isolates in this study are shown. Scale bar represent 0.01.

Figure S1.

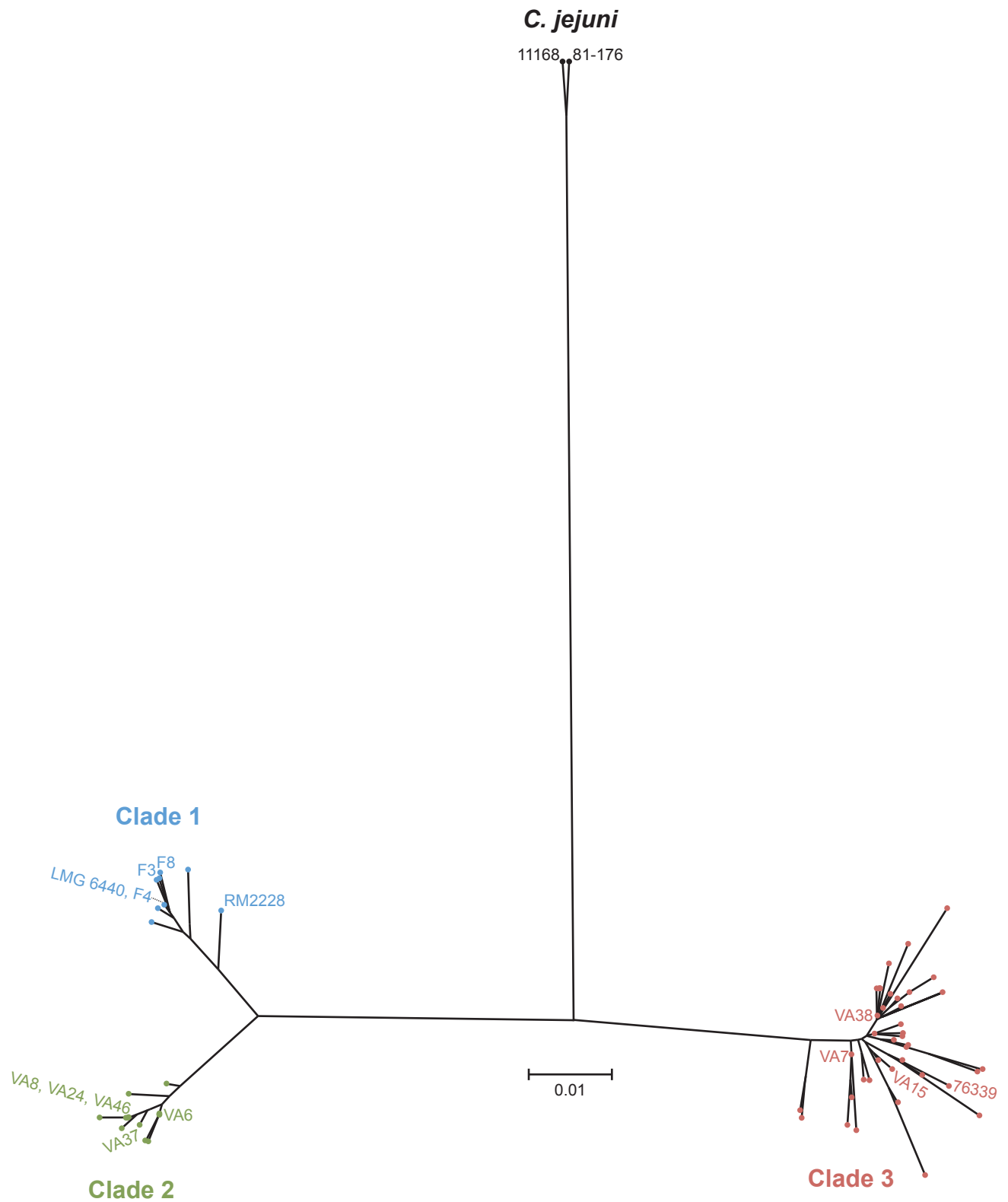


Figure S1. Neighbour-joining tree based on protein sequence alignments of CadF from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Node colours indicate clades; Clade 1 – blue, Clade 2 – green and Clade 3 – red. The exact positions of the isolates in this study are shown. Scale bar represent 0.01.

Figure S2.

| | 10 | 20 | 30 | 40 | 50 |
|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|---------------------------------------|
| <i>C. coli</i> clade 1 | MRKLLLCGL | SSVLF [*] GADNN | VKFEITPTLN | YNYFEGNLD | DNRYAPGIRL |
| <i>C. coli</i> clade 2 | MRKLLLCGL | ASVLF [*] GADNN | VKFEITPTLN | YNYFEGNLD | DNRYAPGIRL |
| <i>C. coli</i> clade 3 | MKLLLCGL | ASVLLGADNN | VKFEITPTLN | YNYFEGNLD | DNRYAPGVRL |
| <i>C. jejuni</i> 11168 | MKIFLCGL | ASVLF [*] GADNN | VKFEITPTLN | YNYFEGNLD | DNRYAPGIRL |
| <i>C. jejuni</i> 81-176 | MKILLLCGL | ASVLF [*] SADNN | VKFEITPTLN | YNYFEGNLD | DNRYAPGIRL |
| | | | | | |
| <i>C. coli</i> clade 1 | GYHFDDFWLD | QLELGLEHYS | DVKYTNSTLT | TDITR [*] TYLSA | IKGIDLGEKF |
| <i>C. coli</i> clade 2 | GYHFDDFWLD | QLELGLEHYS | DVKYTNSTLT | TDITR [*] TYLSA | IKGIDLGEKF |
| <i>C. coli</i> clade 3 | GYHFDDFWLD | QLELGLEHYS | DVKYTNSTLT | TDITR [*] TYLSA | IKGIDLGEKF |
| <i>C. jejuni</i> 11168 | GYHFDDFWLD | QLEF [*] GLEHYS | DVKYTN [*] TNKT | TDITR [*] TYLSA | IKGIDVGEKF |
| <i>C. jejuni</i> 81-176 | GYHFDDFWLD | QLEF [*] GLEHYS | DVKYTN [*] TNKT | TDITR [*] TYLSA | IKGIDVGEKF |
| | | | | | |
| <i>C. coli</i> clade 1 | YFYGLAGGGY | EDFSKGAFDN | KSGGFGHYGA | GLKFRLSDSL | ALRLETRDQI |
| <i>C. coli</i> clade 2 | YFYGLAGGGY | EDFSHGAFDN | KSGGFGHYGA | GLKFRLSDSL | ALRLETRDQI |
| <i>C. coli</i> clade 3 | YFYGLAGGGY | EDFSKGAFDN | KSGGFGHYGA | GLKFRLSDSL | ALRLETRDQI |
| <i>C. jejuni</i> 11168 | YFYGLAGGGY | EDFSNAAYDN | KSGGFGHYGA | GVKFRLSDSL | ALRLETRDQI |
| <i>C. jejuni</i> 81-176 | YFYGLAGGGY | EDFSNAAYDN | KSGGFGHYGA | GVKFRLSDSL | ALRLETRDQI |
| | | | | | |
| <i>C. coli</i> clade 1 | SFHDADHSWV | STLGISFGFG | AKQEKVVVEQ [*] | TKEVVNKPQV [*] | VT [*] PAPAPVVS [*] |
| <i>C. coli</i> clade 2 | SFHDADHSWV | STLGVSFGFG | AKQKVVVEQ [*] | TKEVVSQPQV [*] | VT [*] PAPAPVVS [*] |
| <i>C. coli</i> clade 3 | SFHNA [*] HSWV | STLGISFGFG | AKREKVVAEQ [*] | VKEVAIEPRV [*] | AAPV----- |
| <i>C. jejuni</i> 11168 | NFNHANHNWV | STLGISFGFG | GKKEKAV-E- | --EVADT-R- | ATP----- |
| <i>C. jejuni</i> 81-176 | NFNHANHNWV | STLGISFGFG | GKKEKAV-E- | --EVADT-R- | ATP----- |
| | | | | | |
| <i>C. coli</i> clade 1 | QSKCPEEPRE [*] | GALLDENGCE [*] | KTIYLEGHFD ^{**} | FDKVNINPAF [*] | EEQIKEIAQI [*] |
| <i>C. coli</i> clade 2 | QSKCPAEPRE | GALLDENGCE | KTIYLEGHFD | FDKVNINPAF | EEKIKEIAKI |
| <i>C. coli</i> clade 3 | QSQCPAEPRE | GAML [*] DENGCE | KTISFEGHFG [*] | FDKVDINPVF | EEKIKEIAQI |
| <i>C. jejuni</i> 11168 | QAKCPVEPRE | GALLDENGCE | KTISLEGHFG [*] | FDKTTINPTF | QEKIKEIAKV |
| <i>C. jejuni</i> 81-176 | QAKCPVEPRE | GALLDENGCE | KTISLEGHFG [*] | FDKTTINPTF | QEKIKEIAKV |
| | | | | | |
| <i>C. coli</i> clade 1 | LDENVRYDTI [*] | LEGHTDNIGS | RSYNQKLSER [*] | RANSVAKELE | KFGVDKSRIQ [*] |
| <i>C. coli</i> clade 2 | LDENVRYDTI | LEGHTDNTGS | RSYNQKLSER | RAESVAKELE | KFGVDKSRIQ |
| <i>C. coli</i> clade 3 | LDENARYDTI | LEGHTDNIGS | RAYNQKLSER | RAESVAKELE | KFGVDKDRIQ |
| <i>C. jejuni</i> 11168 | LDENERYDTI | LEGHTDNIGS | RAYNQKLSER | RAKSVANELE | KYGVEKSRIK |
| <i>C. jejuni</i> 81-176 | LDENERYDTI | LEGHTDNIGS | RAYNQKLSER | RAKSVANELE | KYGVEKSRIK |
| | | | | | |
| <i>C. coli</i> clade 1 | TVGYGQDKPR | SSNDTKEGRA | DNRRVEAKFI [*] | LN [*] | |
| <i>C. coli</i> clade 2 | TVGYGQDKPR | SSNDTKEGRA | DNRRVEAKFI | LN | |
| <i>C. coli</i> clade 3 | TVGYGQDKPR | SSNDTKEGRA | DNRRVDAKFI | LR | |
| <i>C. jejuni</i> 11168 | TVGYGQDNPR | SSNDTKEGRA | DNRRVDAKFI | LR | |
| <i>C. jejuni</i> 81-176 | TVGYGQDNPR | SSNDTKEGRA | DNRRVDAKFI | LR | |

Figure S2. Alignment of CadF protein sequences from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Dash; amino acid is missing, grey box; site with variation, asterisk; site where clade 3 isolates differ from clade 1 and 2 isolates, bold; site where clade 3 is identical to *C. jejuni*, box; conserved fibronectin binding site, underlined sequence; OmpA-like domain (amino acids 167-324).

Figure S3.

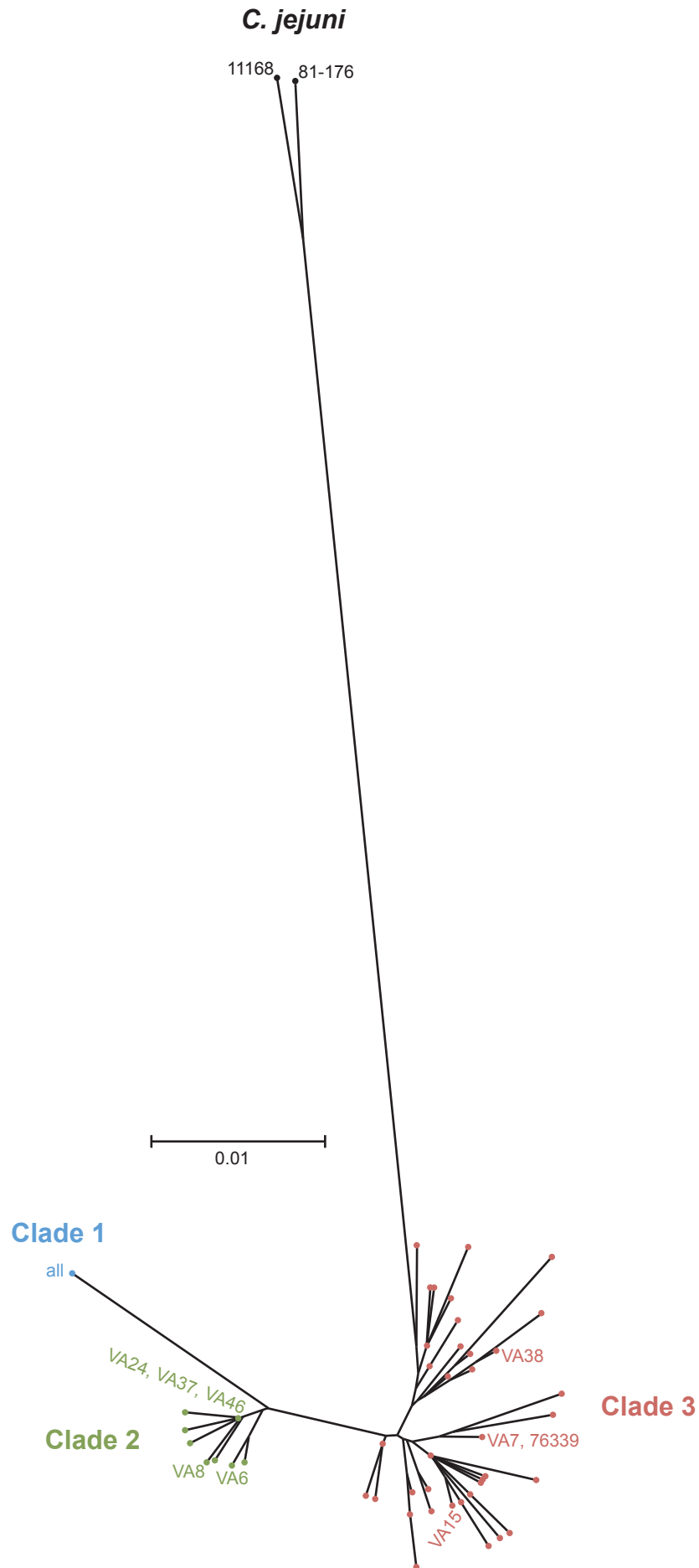


Figure S3. Neighbour-joining tree based on protein sequence alignments of CeuE from *C. coli* clade 1, 2 and 3 isolates (Table S1) together with the *C. jejuni* reference strains 11168 and 81-176. Node colours indicate clades; Clade 1 – blue, Clade 2 – green and Clade 3 – red. The exact positions of the isolates in this study are shown. Scale bar represent 0.01.