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TAXONOMIC DESCRIPTION

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Campylobacter novaezeelandiae sp. nov., isolated from birds and water in New Zealand

Samuel Bloomfield[1]†, David Wilkinson[1], Lynn Rogers[1], Patrick Biggs[1,2], Nigel French[3]‡, Vathsala Mohan[1]§, Matthew Savoian[2], Pierre Venter[4] and Anne Midwinter[1,*]

Abstract

Six isolates of *Campylobacter* with similar non-standard colonial morphologies were identified during studies isolating *Campylobacter* from bird faeces and rivers in New Zealand. Genomic (16S rRNA gene sequencing and whole genome analysis) and phenotypic (MALDI-TOF analysis and conventional biochemical tests) showed that the isolates form a monophyletic clade with genetic relationships to *Campylobacter coli*/*Campylobacter jejuni* and *Campylobacter peloridis*/*Campylobacter amoricus*. They may be distinguished from other *Campylobacter* by their MALDI-TOF spectral pattern, their florid α-haemolysis, their ability to grow anaerobically at 37 °C, and on 2% NaCl nutrient agar, and their lack of hippuricase. This study shows that these isolates represent a novel species within the genus *Campylobacter* for which the name *Campylobacter novaezeelandiae* sp. nov. is proposed. The presence of *C. novaezeelandiae* in water may be a confounder for freshwater microbial risk assessment as they may not be pathogenic for humans. The type strain is B423b^T (=NZRM 4741^T=ATCC TSD-167^T).

The genus *Campylobacter* was described by Sebald and Veron [1] while reclassifying the genus *Vibrio*s. Since then, 32 *Campylobacter* species with nine subspecies have been identified [2]. Campylobacteriosis is one of the most common bacterial gastrointestinal illnesses of humans in the developed world [3], including in New Zealand, which has had a high notification rate [4].

Campylobacter are frequently associated with the gastrointestinal tracts of birds [5], particularly chickens [6], but have also been detected in wild birds ranging from albatross [7] to zebra finch [8]. In New Zealand, *Campylobacter* has also been isolated frequently from waterways, with the majority of isolates being attributed to wild bird sources [9, 10].

ISOLATION AND ECOLOGY

As part of source attribution and population structure studies on *Campylobacter* [11, 12], six isolates with dark-field microscopy characteristics of *Campylobacter* but non-standard agar-plate morphology were isolated from Bolton broth enrichments (LabM) subcultured onto modified charcoal cefoperazone (mCCDA) agar (Fort Richard Laboratories) after 48 h of incubation. Two isolates were from rivers (100 ml water was filtered through a 0.45 µm filter, and the filter was placed in Bolton broth). Four isolates were from wild non-native bird faeces (three starlings and one duck) collected from deposited droppings with a swab which was placed in Bolton broth with subculture to mCCDA agar after 48 h incubation. Initial enrichment and growth were performed in a microaerobic atmosphere (10% CO_2, 5% O_2, 85% N_2) in a variable atmosphere incubator (Don Whitley) at 42 °C. Single colonies were subcultured to Columbia horse blood agar

Author affiliations: [1]Molecular Epidemiology and Public Health Laboratory, School of Veterinary Science, Massey University, Palmerston North, New Zealand; [2]School of Fundamental Sciences, Massey University, Palmerston North, New Zealand; [3]Infectious Disease Research Centre, Hopkirk Research Institute, Massey University, Palmerston North, New Zealand; [4]Fonterra Research and Development Centre, Palmerston North, New Zealand.
***Correspondence:** Anne Midwinter, a.c.midwinter@massey.ac.nz
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Abbreviations: CDSs, coding-DNA sequences; mCCDA, modified charcoal cefoperazone agar; TSI, triple sugar iron agar; TTC, 2,3,5 triphenyltetrazolium.
†Present address: Quadram Institute Bioscience, Norwich, Norfolk, UK
‡Present address: New Zealand Food Safety and Science Research Centre, Massey University, Palmerston North, New Zealand
§Present address: Livestock Improvement Corporation, Hamilton, New Zealand.
Genome assemblies have been deposited in GenBank under the accession numbers QPGR00000000, QPGQ00000000 and QPGP00000000, and read data are deposited under SRA codes SRR8367113, SRR8367114 and SRR8367115 within the BioProject PRJNA480171. 16S rRNA gene sequences have been deposited in GenBank under the accession numbers MK791738 - MK791743.
One supplementary table and one supplementary figure are available with the online version of this article.

Table 1. Characteristics of the Campylobacter *novaezeelandiae* sp. nov. isolates described in this study

Isolate	Source	Location	Isolation date	16S rRNA gene accession	Genome accession
B423b^T	Mallard duck	Palmerston North, New Zealand	Aug 2008	MK791741	QPGR00000000
B571b	Starling	Palmerston North, New Zealand	Sept 2008	MK791743	–
B716b	Starling	Palmerston North, New Zealand	Nov 2008	MK791742	–
B1491	Starling	Palmerston North, New Zealand	May 2009	MK791739	QPGQ00000000
W441b	Oroua River	Feilding, New Zealand	Oct 2008	MK791738	–
W677a	Pareora River	South Canterbury, New Zealand	Apr 2011	MK791740	QPGP00000000

(Fort Richard Laboratories) before storage in 15% glycerol in nutrient broth no. 2 (Oxoid) at −80 °C. Interestingly, we have not found similar isolates from chickens [12], ruminants [13], dogs and cats [14, 15] or, most importantly, from human cases of campylobacteriosis [12], which suggests that these isolates have a restricted host range and low virulence.

In New Zealand, freshwater quality standards are based around the use of *E. coli* counts as an indicator relating to *Campylobacter* presence [16] and thus human health risk. The presence of *Campylobacter novaezeelandiae* in river water may act as a confounder for this assumption, requiring modification of the quantitative microbial risk assessments.

Characteristics of the isolates are described in Table 1.

16S rRNA GENE PHYLOGENY

16S rRNA gene PCR analyses were performed using crude DNA extracted by boiling [17], the primers and conditions described by Lane [18] with Sanger sequencing of the products at the Massey Genome Service (Massey University). Comparison of the sequences suggested that all the isolates were related and formed a single distinct species previously described as '*Campylobacter* sp. nov. 3' [10]. A 16S rRNA gene-based phylogeny was reconstructed from the 16S rRNA loci extracted from the whole genome sequenced isolates [19] using barrnap (https://github.com/tseemann/barrnap), and aligned using MAFFT version 7 [20]. The resulting phylogenetic tree (Fig. 1) shows that the isolates (henceforth called

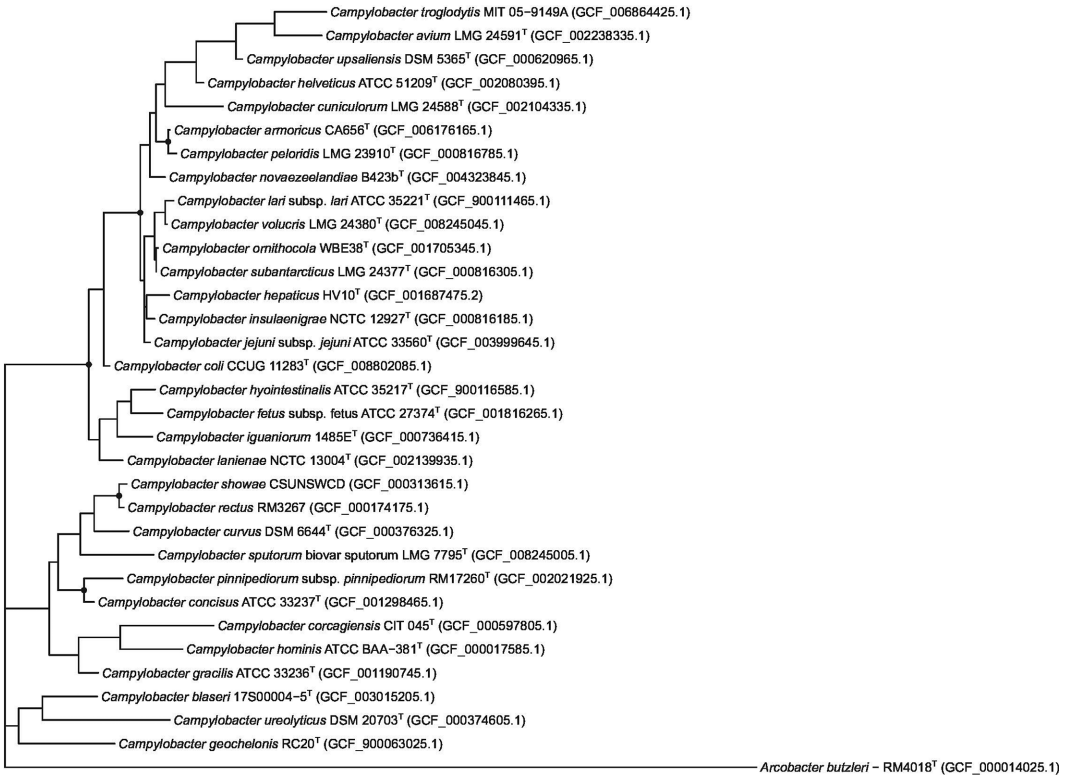

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences. The dots on the nodes represent nodes with bootstrap support >90%.

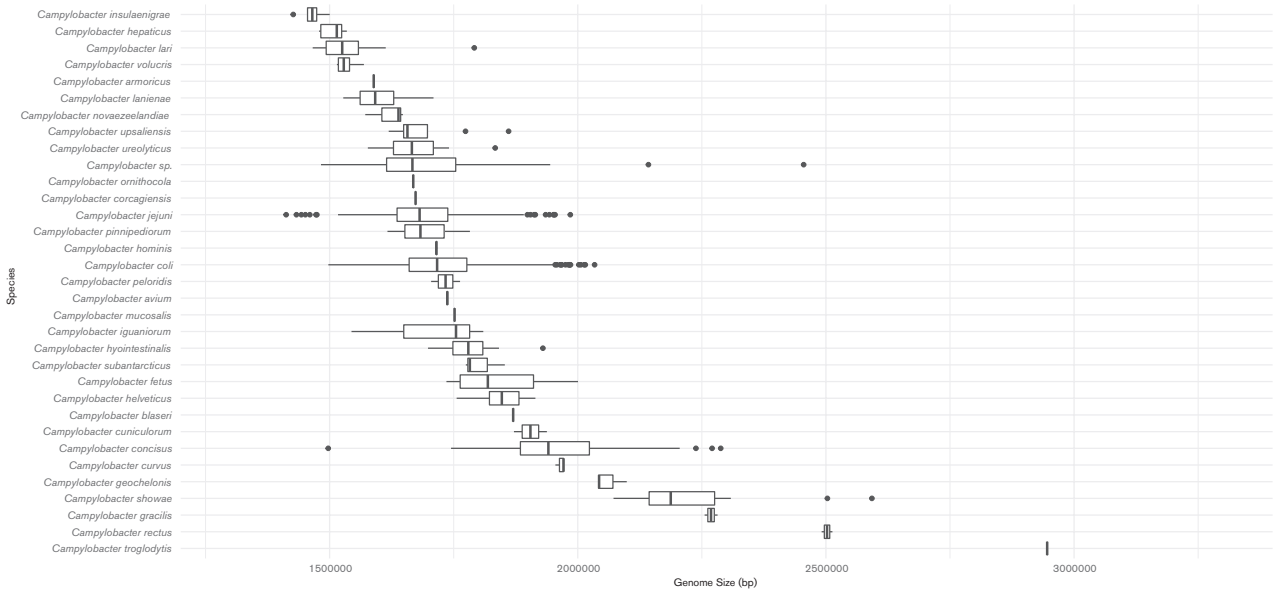

Fig. 2. Comparison of the genome size of Campylobacter *novaezeelandiae* sp. nov. and other *Campylobacter* species

Campylobacter novaezeelandiae sp. nov.) clustered together and were closest to *Campylobacter amoricus* and *Campylobacter peloridis*. Sequence alignment showed the 16S rRNA genes of the *C. novaezeelandiae* sp. nov. isolates to be on average 97.6% similar to those of *C. amoricus* and 97.4% similar to those of *C. peloridis*. As a check of the quality of the genome sequence [21], the sequences of the Sanger sequenced 16S rRNA ene PCR products and the those extracted from the whole genome sequences were compared. The whole genome 16S genes all contained a thymidine insertion at the same position compared to the 16S rRNA PCR products. *Campylobacter* species contain three copies of the 16S rRNA genes [22] and the 16S gene assembled is a combination of the three, whilst PCR targets only one of the genes, suggesting that one or both of the other 16S genes contain this insertion and explaining the differences between the 16S rRNA gene sequences. Apart from this insertion, the 16S rRNA gene sequences generated by PCR and whole genome sequencing were identical.

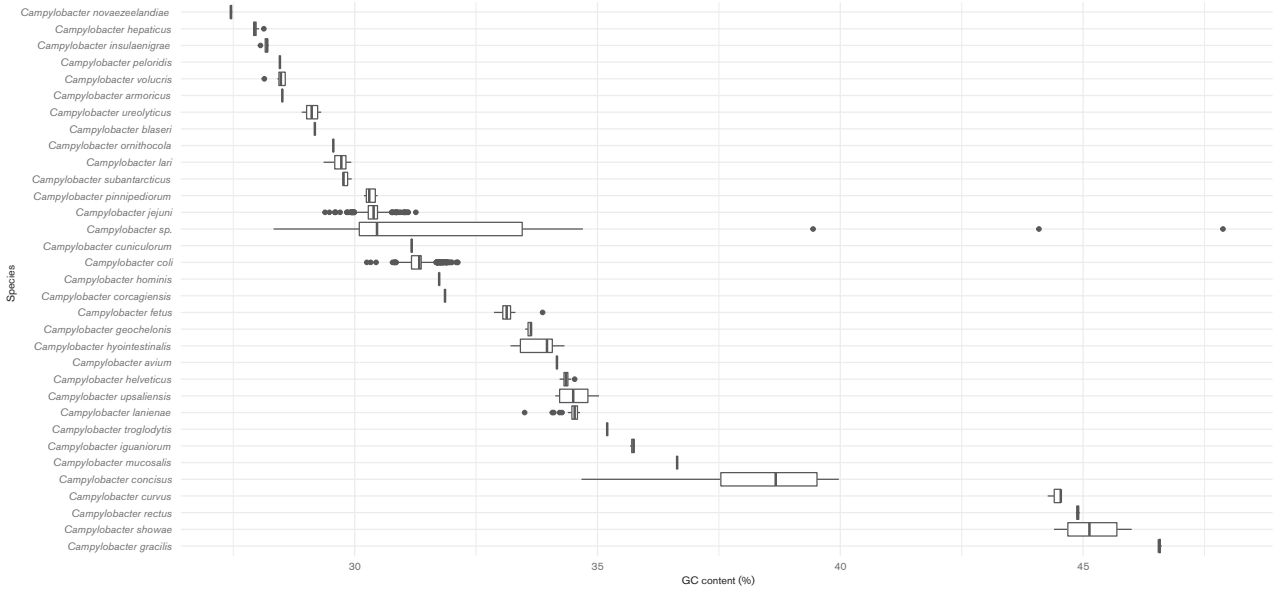

Fig. 3. Comparison of the G+C content of Campylobacter *novaezeelandiae* sp. nov. and other *Campylobacter* species.

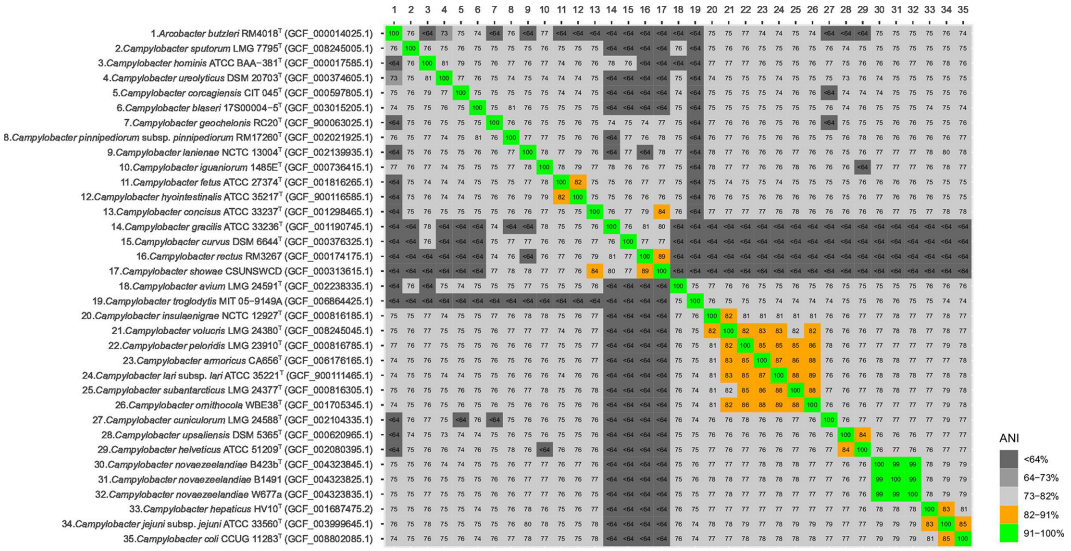

Fig. 4. Average nucleotide identity (ANI) values (%) comparing Campylobacter *novaezeelandiae* sp. nov. and other *Campylobacter* taxa. The list of accession numbers is in Table S1 (available in the online version of this article).

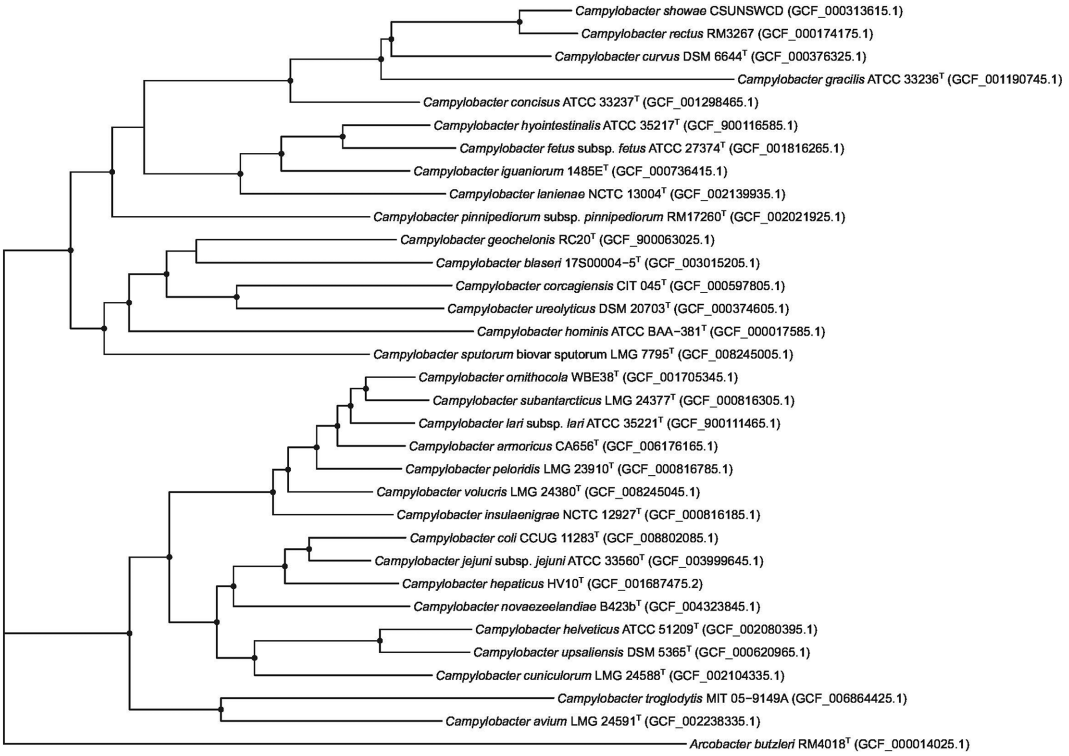

Fig. 5. Maximum-likelihood tree based on the core genome. The dots on the nodes represent nodes with bootstrap support >90%.

GENOME FEATURES

Whole genomes of three of the isolates were sequenced, assembled and examined as described previously [19]. The genomic DNA G+C content of the strains was estimated at 27.5mol% using the Geneious R10 software package [23]. Comparisons of genome size and G+C content were performed relative to 3210 genomes available in the NCBI RefSeq database that are assigned to the genus '*Campylobacter*'. Genomes were downloaded using the NCBI genome download tool (available at https://github.com/kblin/ncbi-genome-download).

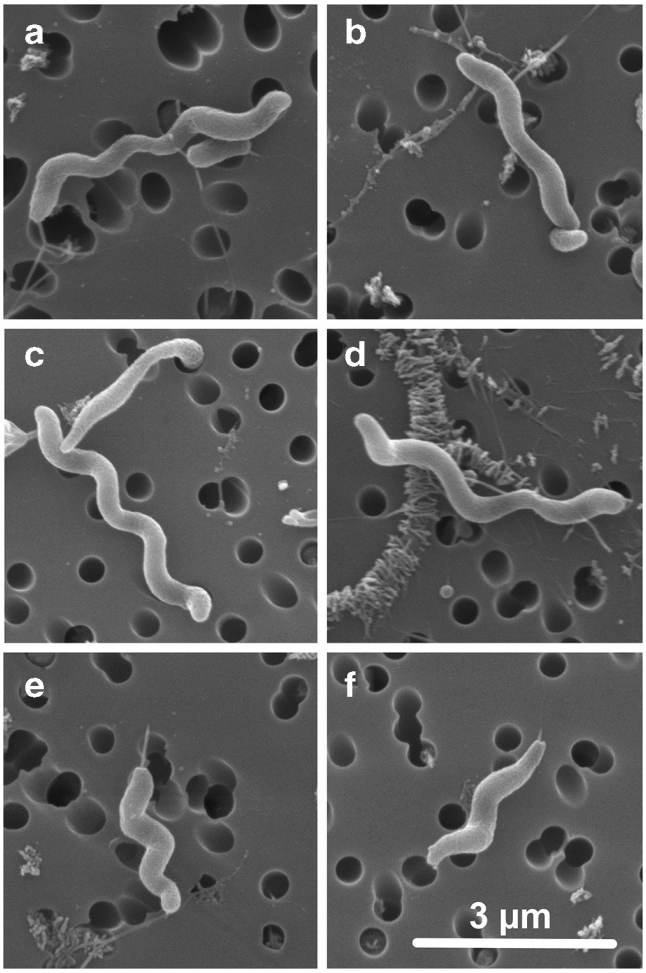

Fig. 6. Electron micrograph of Campylobacter *novaezeelandiae* sp. nov.

NCBI-assigned species taxon identifiers were used to group the data. Analysis and visualizations were performed in RStudio. The genome sizes of the *C. novaezeelandiae* isolates fell within the range of *Campylobacter* (Fig. 2), however the *C. novaezeelandiae* isolates possessed the lowest G+C content of all current members of the genus *Campylobacter* [24, 25] (Fig. 3).

Genomic average nucleotide identity (ANI) values were calculated using an ANI calculator [26] showing that the values between the isolates of *C. novaezeelandiae* were 99.0–100% while the most similar of the other *Campylobacter* species was *Campylobacter jejuni* with 79.7% ANI similarity (Fig. 4).

Prokka version 1.14.5 [27] was used to annotate the three draft genomes. Roary version 3.11.2 [28] was used to cluster the annotated sequences and investigate the pan-genome, a clustering similarity threshold of 70% was specified in this analysis in order to allow clustering of gene sequences that were divergent at the genus level. The three *C. novaezeelandiae* isolates contained between 1485 and 1591 coding-DNA sequences (CDSs). They shared a pan-genome of 1781 CDSs: 1395 (78%) of which were core and 386 (21%) of which were accessory. Even though this is a small sample size, it demonstrates significant variation in gene content between isolates of this species.

ARIBA version 2.14.1 [29] was used to investigate if the three genomes contained antimicrobial resistance genes using the ResFinder database [30], plasmids using the PlasmidFinder database [31], or virulence genes using the VFDB core database [32]. No AMR genes or plasmids were identified amongst the genomes, but one virulence gene (*Cj1427c*) was found within the genome of the type strain (B423b^T). This gene encodes a sugar-nucleotide epimerase/dehydratase involved in capsular formation [33]. The reference databases used for this analysis consist of genes and genetic markers from described bacterial species, *C. novaezeelandiae* may contain novel or distantly related AMR genes, plasmids and virulence factors not yet described in these databases. For phylogenetic analyses, genome sequences derived from species type strains (as specified by https://lpsn.dsmz.de/genus/campylobacter) were used where available. If unavailable, RefSeq representative genomes were used. Genomes from a total of 32 *Campylobacter* species were used. *Campylobacter mucosalis* was excluded from the analysis, as RefSeq indicated that the only available genome from this species contained significant contamination. Gene clustering in Roary identified 79 core genes (and 188 soft-core genes [occurring in 95% of the genomes]) that were conserved across the genus *Campylobacter*.

Phylogenies from alignments of the core gene sequences were generated in MEGA-X [34], using the maximum-likelihood method and the general time-reversible model. A discrete gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter=0.7929)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 19.23% sites). Trees with the highest log likelihood were chosen for display, and 100 bootstrap replicates were performed to estimate node support. Trees were visualized using 'ggtree' [35] in RStudio [36]. In contrast to the 16S rRNA gene sequence similarities (Fig. 1), and in accordance with whole genome ANI analysis (Fig. 4), phylogenetic analysis based on core gene contents supported a close relationship to *C. cuniculorum*, *C. upsaliensis*, *C. helveticus*, *C. hepaticus*, *C. coli* and *C. jejuni* (Fig. 5).

PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

MALDI-TOF protein spectra were obtained as described by Koziel *et al.* [37] and discriminant peaks between species were identified using the 'MALDIquant' [38] 'sda' [39] and 'crossval' [40] R packages. *C. jejuni* subsp. *jejuni* NCTC 11168, *C. coli* ATCC 33559^T, *C. hyointestinalis* ATCC 33560^T, *C. fetus* subsp *fetus* ATCC 27374^T and *C. upsaliensis* ATCC 43954^T (ESR Culture Collection) were used as controls. Representative averaged MALDI-TOF spectra and significantly discriminant peaks for different species are shown in Fig. S1.

Table 2. Phenotypic characteristics of Campylobacter *novaezeelandiae* sp. nov. as compared to other *Campylobacter* taxa

Taxa: 1, *Campylobacter novaezeelandiae* sp. nov. (*n*=6); 2, *C. armoricus*; 3, *C. blaseri*; 4, *C. avium*; 5, *C. canadensis*; 6, *C. coli*; 7, *C. concisus* ; 8, *C. corcagiensis*; 9, *C.cuniculorum*; 10, *C. curvus*; 11, *C. fetus* subsp. *fetus*; 12, *C. fetus* subsp. *testudinum*; 13, *C. fetus* subsp. *venerealis*; 14, *C. geochelonis*; 15, *C. gracilis*; 16, *C. helveticus*; 17, *C. hepaticus*; 18, *C. hominis*; 19, *C. hyointestinalis* subsp. *hyointestinalis*; 20, *C. hyointestinalis* subsp. *lawsonii*; 21, *C. iguaniorum*; 22, *C. insulaenigrae*; 23, *C.jejuni* subsp. *doylei*; 24, *C. jejuni* subsp. *jejuni*; 25, *C. lanienae*; 26, *C. lari* subsp. *concheus*; 27, *C. lari* subsp. *lari*; 28, *C. mucosalis*; 29, *C. ornithocola*; 30, *C. peloridis*;31, *C. pinnipediorum* subsp. *caledonicus*; 32, *C. pinnipediorum* subsp. *pinnipediorum*; 33, *C. rectus*; 34, *C. showae*; 35, *C. sputorum*; 36, *C. subantarcticus*; 37, *C.upsaliensis*; 38, *C. ureolyticus*; 39, *C. volucris*. +, All strains examined give a positive result; –, strains examined give a negative result; U, unknown; F, 7–28% strains positive; V, 29–69% strains positive; M, 70–95% strains positive; D, unable to be determined; w, weak.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Motility	+		–	+	+	+	+	–	+	+	+	+	+	+	–	+	+	–	+	+
Oxidase	+	+	+	+	+	+	V	+	+	+	+	+	+	+	–	+	+	+	+	+
Catalase	+	+	+	w	V	+	–	+	+	–	+	+	M	+	V	–	+	–	+	+
Urease	–	+	+	–	V	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Nitrate reduction	+	–	+	+	V	+	F	M	+	+	+	+	M	+	M	+	V	–	+	+
Hippurate hydrolysis	–	–	–	+	–	–	–	–	–	F	–	–	–	+	–	–	M	–	–	–
Indoxyl acetate hydrolysis	+	–	+	+	–	+	–	V	+	V	–	–	–	–	M	+	+	–	–	–
Alkaline phosphatase	–	–	+	–	–	–	M	+	–	V	–	–	–	–	–	–	U	–	–	F
H_2S production TSI	–	U	+	–	V	–	–	+	–	F	–	–	–	–	–	–	–	–	+	+
Alpha haemolysis	+	–	–	–	–	F	F	–	+	F	–	–	V	–	–	+	–	–	V	F
Growth 25 °C microaerobic	–	–	+	–	–	–	–	U	–	–	+	+	+	+	–	–	–	–	F	–
Growth 37 °C microaerobic	+	+	+	+	+	+	+	+	+	V	+	+	+	+	–	+	+	–	+	+
Growth 42 °C microaerobic	+	+	+	+	+	+	M	+	M	V	M	V	–	–	M	+	+	–	+	+
Growth 37 °C anaerobic	+	+	+	–	+	–	+	+	–	+	F	+	M	+	+	–	–	+	–	w
Growth 37 °C aerobic	–	–	–	–	–	–	–	–	–	–	–	V	–	–	–	–	–	–	–	–
Growth on nutrient agar	+	U	U	–	–	+	F	w	+	+	M	U	M	U	+	M	+	U	+	+
Growth on MacConkey	–	U	+	–	+	V	–	–	–	M	M	M	V	–	M	–	–	–	V	V
R to nalidixic acid (30 µg)	-*	–	–	–	V	–	M	+	V	+	+	+	V	+	V	–	V	V	+	+
R to cephalothin (30 µg)	+	R	–	+	–	+	–	–	M	–	–	U	–	–	–	–	M	–	F	–
H_2 requirement	–	–	–	V	–	–	+	–	–	+	–	–	–	–	+	–	–	+	–	U
DNA G+C content (mol%)	27.4	28	29	35	U	31	37–41	31.9	32.4	45–46	33–35	33	33–34	33.6	44–46	34	27.9	32–33	34–36	31–33
Selenite reduction	–	V	U	–	U	+	F	U	–	–	M	U	F	–	–	–	U	–	+	+
Growth on 2% NaCl	+	–	U	–	–	–	F	+	–	V	–	U	F	+	–	F	–	U	–	–
Growth on 1% glycine	+	+	w	–	V	M	F	+	–	+	+	+	F	+	+	V	+	+	+	F
Growth on 0.04% TTC	F	V	U	+	U	+	–	–	V	+	–	+	–	–	–	–	+	–	F	–
TTC reduction	F	V	U	–	U	+	–	–	V	M	–	+	–	–	–	–	U	–	F	–

Continued

Table 2. Continued

Characteristic	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Motility	U	+	+	+	+	+	+	+	U	U	U	U	+	+	+	+	+	–	+
Oxidase	+	+	+	+	+	+	+	+	–	+	+	+	+	V	+	+	+	+	+
Catalase	+	+	M	+	+	+	+	–	+	+	–	+	F	+	V†	+	–	F	+
Urease	–	–	–	–	–	–	–	–	+	U	+	+	–	–	V‡	U	–	+	U
Nitrate reduction	+	+	–	+	+	+	+	F	V	U	+	+	+	+	M	U	+	+	+
Hippurate hydrolysis	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Indoxyl acetate hydrolysis	–	–	+	+	–	U	F	–	–	U	–	–	+	V	–	–	+	V	–
Alkaline phosphatase	U	U	–	–	+	U	–	–	–	U	U	U	–	–	–	U	–	–	–
H_2S production TSI	+	–	–	–	–	U	–	+	–	U	+	+	–	V	+	–	–	–	–
Alpha haemolysis	+	ND	+	+	+	U	M	F	–	U	+	+	+	+	+	+	+	V	U
Growth 25 °C microaerobic	+	–	–	–	–	–	–	–	–	U	+	+	–	–	–	–	–	–	–
Growth 37 °C microaerobic	+	+	+	+	+	+	+	+	+	+	+	+	–	V	+	+	+	+	+
Growth 42 °C microaerobic	–	–	–	+	+	+	+	+	+	+	–	–	w	V	M	+	M	V	+
Growth 37 °C anaerobic	w	–	–	–	w	U	–	+	+	U	+	+	+	+	+	+	–	+	+
Growth 37 °C aerobic	–	–	–	–	–	–	–	–	–	U	–	–	–	–	–	–	–	–	–
Growth on nutrient agar	U	+	+	+	U	+	+	+	U	+	U	U	F	V	M	–	+	M	–
Growth on MacConkey	U	U	–	–	U	U	U	M	U	U	U	U	–	+	V	F	–	V	w
R to nalidixic acid (30 µg)	+	+	–	–	+	–	M	M	U	M	–	–	M	–	M	+	–	–	+
R to cephalothin (30 µg)	–	+	–	+	+	+	+	–	U	F	–	–	–	–	–	–	F	–	+
H_2 requirement	–	–	–	–	U	U	–	+	–	U	–	–	+	+	–	U	–	+	–
DNA G+C content (mol%)	36	U	28–30	30–31	36	30	31–33	38–39	29.5	29	31	30	45–46	44–46	30–33	30	32–35	28–30	29
Selenite reduction	U	U	–	M	U	U	V	F	U	U	U	U	–	–	U	–	+	–	+
Growth on 2% NaCl	U	–	–	–	–	+	+	–	U	M	U	U	M	+	+	+	–	+	–
Growth on 1% glycine	+	+	F	M	–	+	+	V	+	+	–	V	M	V	+	M	+	+	–
Growth on 0.04% TTC	U	U	V	M	U	U	M	–	U	U	U	U	–	–	–	U	–	+	–
TTC reduction	U	U	V	M	U	U	M	–	U	U	U	U	–	–	–	U	–	–	–

*Zone diameters varied around 18–19 mm. *C. novaezeelandiae* does not grow well on Mueller–Hinton agar containing either sheep or horse blood.
†Biovar fecalis strains produce catalase.
‡ Biovar paraureolyticus strains produce urease.

Electron microscopy was performed as described in Wheeler *et al.* [41] using an FEI Quanta 200 scanning electron microscope. All isolates were spiral-shaped rods (Fig. 6).

Galleria mellonella (Biosuppliers) larvae were challenged with the *C. novaezeelandiae* isolates W441b and W667a as described by Champion *et al.* [42] using 31G insulin syringes (Becton Dickinson) for inoculation, with incubation at 37 °C in a variable atmosphere incubator with H_2 (5% H_2, 10% CO_2, 3% O_2, 82% N_2). Nine isolates of *C. jejuni* isolated from invasive campylobacteriosis in humans and described in Wheeler *et al.* [41] were used as positive controls, phosphate-buffered saline inoculated and un-inoculated caterpillars were included as negative controls. Analyses were performed in RStudio, using the 'survival' (https://github.com/therneau/survival) and 'survminer' (https://github.com/kassambara/survminer) packages. Larvae inoculated with *C. novaezeelandiae* showed lower mortality than those inoculated with the *C. jejuni* controls (P=0.0089). The observed relative hazard ratio of 0.53 suggested that isolates of *C. novaezeelandiae* are approximately half as virulent as isolates of *C. jejuni* in the wax moth caterpillar model. This ability to kill larvae is discordant with our observation that *C. novaezeelandiae* has not been isolated from cases of campylobacteriosis and suggest that *C. novaezeelandiae* may be a useful model organism for exploring the *G. mellonella* virulence model.

Time taken for transformation to coccoidal forms was performed as described [43] using Columbia horse blood agar in a microaerobic atmosphere at 42 °C. After 3 days of incubation most (80–90%) of the bacteria are rod-shaped, but after 6 days of incubation the proportion of rod-shaped bacteria has decreased to between 10 to 30% with the remainder of the bacteria being coccoidal. Gram staining was performed as described by Chapin and Lauderdale [44]. Motility was observed by dark-field microscopy. Tests for oxidase, catalase, motility, hippurate hydrolysis and nitrate reduction were performed as described by MacFaddin [45]. Alkaline phosphatase activity was tested using an API Campy kit (bioMérieux). Indoxyl acetate hydrolysis was tested with the modification described by On and Holmes [46]. Hydrogen sulphide production was tested as described by Ma *et al.* [47]. Cephalothin and nalidixic acid sensitivity assays were performed using EUCAST zone cut-offs [48] on both sheep and horse blood-containing Mueller–Hinton agar (Fort Richard Laboratories). Temperature and atmosphere requirements were examined as described by On and Holmes [49] on Columbia horse blood agar and using atmospheres provided by variable atmosphere incubators or gas-generating envelopes (Oxoid). Growth on MacConkey agar (Fort Richard Laboratories), nutrient agar no. 2 (Oxoid), nutrient agar plus 1% w/v glycine and nutrient agar plus 2% w/v NaCl was as described by On and Holmes [49]. Growth on and reduction of 0.1% w/v selenite and 0.04% w/v 2,3,5 triphenyltetrazolium (TTC) in nutrient agar no. 2 were as described by On and Holmes [49]. Unless otherwise mentioned, tests were performed at 37 °C in a variable atmosphere incubator with H_2 (5% H_2, 10% CO_2, 3% O_2, 82% N_2). Control organisms were *C. jejuni* subsp. *jejuni* NCTC 11168, *C. jejuni* NCTC 11351^T, *C. coli* ATCC 33559^T, *C. hyointestinalis* ATCC 33560^T, *C. fetus* subsp *fetus* ATCC 27374^T, *C. upsaliensis* ATCC 43954^T, *C. lanienae* LR283 and *C. helveticus* CCUG 30683. Characteristics are given in Table 2, and phenotypic descriptions are given below.

DESCRIPTION OF *CAMPYLOBACTER NOVAEZEELANDIAE* SP. NOV.

Campylobacter novaezeelandiae (no.vae.zee.lan'di.ae. N.L. gen. n. novaezeelandiae of Nova Zeelandia, pertaining to New Zealand).

Cells are Gram-negative, motile, spiral rods, 3–4 µm long. After 2 days incubation on Columbia horse blood agar at 37 °C in a microaerobic atmosphere containing hydrogen gas, colonies are small (0.5–1 mm), grey, smooth and entire with slightly convex surfaces. Strong, dark α-haemolysis is seen on Columbia horse blood agar. A distinctive '*Campylobacter*' odour [50] is produced. On mCCDA agar, colonies are small and grey with irregular margins. Isolates will grow microaerobically (without hydrogen) at 42 °C, but growth was faster at 37 °C. They grow anaerobically at 37 °C but do not grow in ambient air at 37 °C. They grow on nutrient agar, nutrient agar +1% w/v glycine and nutrient agar +2% w/v NaCl, but not on MacConkey agar or on nutrient agar with 0.1% w/v selenite. Only 1/6 isolates grew on nutrient agar containing 0.04% w/v tetrazolium chloride, that isolate was also capable of reducing tetrazolium. They grow poorly on Mueller–Hinton agar containing either sheep or horse blood. Isolates are motile and positive for oxidase, catalase, indoxyl acetate hydrolysis and nitrate reduction. They do not hydrolyse hippurate, produce H_2S in TSI, show alkaline phosphatase activity nor do they produce urease. All isolates are resistant to cephalothin and sensitive to nalidixic acid. Pathogenicity for vertebrates is unknown and we have not isolated this species from humans or symptomatic animals [12]; however, they were capable of causing death in the *Galleria mellonella* larval model. The type strain is B423b^T (=NZRM 4741^T=ATCC TSD-167^T). The GenBank accession numbers for the genome assemblies of B423b^T, B1491 and W677a are QPGR00000000, QPGQ00000000 and QPGP00000000, respectively, while the read data are deposited under SRA codes SRR8367113, SRR8367114 and SRR8367115 within the BioProject PRJNA480171. The 16S rRNA gene sequences have been deposited in GenBank under the accession numbers MK791738–MK791743.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Sebald M, Veron M. Teneur en bases de l'ADN et classification des vibrions. *Ann Inst Pasteur* 1963;105:897–910.
2. Gilbert MJ, Zomer AL, Timmerman AJ, Spaninks MP, Rubio-García A *et al*. *Campylobacter blaseri* sp. nov., isolated from common seals (*Phoca vitulina*). *Int J Syst Evol Microbiol* 2018;68:1787–1794.
3. Samuel MC, Vugia DJ, Shallow S, Marcus R, Segler S *et al*. Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996–1999. *Clin Infect Dis* 2004;38:S165–S174.
4. Baker M, Wilson N, Edwards R. *Campylobacter* infection and chicken: an update on New Zealand's largest 'common source outbreak'. *N Z Med J* 2007;120:75–79.
5. Waldenström J, Broman T, Carlsson I, Hasselquist D, Achterberg RP *et al*. Prevalence of *Campylobacter jejuni*, *Campylobacter lari*, and *Campylobacter coli* in different ecological guilds and taxa of migrating birds. *Appl Environ Microbiol* 2002;68:5911–5917.
6. Newell DG, Fearnley C. Sources of *Campylobacter* colonization in broiler chickens. *Appl Environ Microbiol* 2003;69:4343–4351.
7. Debruyne L, Broman T, Bergström S, Olsen B, On SLW *et al*. *Campylobacter subantarcticus* sp. nov., isolated from birds in the sub-Antarctic region. *Int J Syst Evol Microbiol* 2010;60:815–819.
8. Benskin CMH, Rhodes G, Pickup RW, Wilson K, Hartley IR. Diversity and temporal stability of bacterial communities in a model passerine bird, the zebra finch. *Mol Ecol* 2010;19:5531–5544.
9. Carter PE, McTavish SM, Brooks HJL, Campbell D, Collins-Emerson JM *et al*. Novel clonal complexes with an unknown animal reservoir dominate *Campylobacter jejuni* isolates from river water in New Zealand. *Appl Environ Microbiol* 2009;75:6038–6046.
10. French N, Yu S, Biggs P, Holland B, Fearnhead P *et al*. Evolution of Campylobacter species in New Zealand. In: Sheppard SK (editor). *Campylobacter Ecology and Evolution*. Caister Academic Press; 2014. pp. 221–240.
11. Mohan V, Stevenson M, Marshall J, Fearnhead P, Holland BR *et al*. *Campylobacter jejuni* colonization and population structure in urban populations of ducks and starlings in New Zealand. *Microbiologyopen* 2013;2:659–673.
12. Mullner P, Shadbolt T, Collins-Emerson JM, Midwinter AC, Spencer SEF *et al*. Molecular and spatial epidemiology of human campylobacteriosis: source association and genotype-related risk factors. *Epidemiol Infect* 2010;138:1372–1383.
13. Wilkinson DA, O'Donnell AJ, Akhter RN, Fayaz A, Mack HJ *et al*. Updating the genomic taxonomy and epidemiology of *Campylobacter hyointestinalis*. *Sci Rep* 2018;8:2393.
14. Bojanić K, Midwinter AC, Marshall JC, Biggs PJ, Acke E *et al*. Isolation of emerging *Campylobacter* species in working farm dogs and their frozen home-killed raw meat diets. *J Vet Diagn Invest* 2019;31:23–32.
15. Bojanić K, Midwinter AC, Marshall JC, Rogers LE, Biggs PJ *et al*. Isolation of *Campylobacter* spp. from client-owned dogs and cats, and retail raw meat pet food in the Manawatu, New Zealand. *Zoonoses Public Health* 2017;64:438–449.
16. McBride G, Till D, Ryan T, Ball A, Lewis G *et al*. Pathogen occurence and human health risk assessment analysis 2002.
17. Nohra A, Grinberg A, Midwinter AC, Marshall JC, Collins-Emerson JM *et al*. Molecular epidemiology of *Campylobacter coli* strains isolated from different sources in New Zealand between 2005 and 2014. *Appl Environ Microbiol* 2016;82:AEM.00934-16.
18. Lane DJ. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (editors). *Nucleic Acid Techniques in Bacterial Systematics*. Chicester: John Wiley & Sons; 1991. pp. 115–175.
19. Wilkinson DA, Midwinter AC, Kwan E, Bloomfield SJ, French NP *et al*. Draft whole-genome sequences of three isolates of a novel strain of a *Campylobacter* sp. isolated from New Zealand birds and water. *Microbiol Resour Announc* 2019;8:1–2.
20. Katoh K, Rozewicki J, Yamada KD. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform* 2019;20:1160–1166.
21. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
22. Taylor DE, Eaton M, Yan W, Chang N. Genome maps of *Campylobacter jejuni* and *Campylobacter coli*. *J Bacteriol* 1992;174:2332–2337.
23. Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A *et al*. Geneious.
24. Debruyne L, Gevers D, Vandamme P. Taxonomy of the family *Campylobacteraceae*. In: Nachamkin I, Szymanski C, Blaser M (editors). *Campylobacter*. Washington, DC, USA: ASM Press; 2008. pp. 3–26.
25. Van TTH, Elshagmani E, Gor MC, Scott PC, Moore RJ. *Campylobacter hepaticus* sp. nov., isolated from chickens with spotty liver disease. *Int J Syst Evol Microbiol* 2016;66:4518–4524.
26. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P *et al*. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
27. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
28. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S *et al*. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 2015;31:3691–3693.
29. Hunt M, Mather AE, Sánchez-Busó L, Page AJ, Parkhill J *et al*. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genom* 2017;3:1–11.
30. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S *et al*. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012;67:2640–2644.
31. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O *et al*. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;58:3895–3903.
32. Chen L, Yang J, Yu J, Yao Z, Sun L *et al*. VFDB: a reference database for bacterial virulence factors. *Nucleic Acids Res* 2005;33:D325–D328.
33. Wong A, Lange D, Houle S, Arbatsky NP, Valvano MA *et al*. Role of capsular modified heptose in the virulence of *Campylobacter jejuni*. *Mol Microbiol* 2015;96:1136–1158.
34. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
35. Yu G, Smith DK, Zhu H, Guan Y, Lam Tommy Tsan-Yuk, Lam TT. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol Evol* 2017;8:28–36.
36. RStudio Team. RStudio: integrated development environment for R 2015.
37. Koziel M, O'Doherty P, Vandamme P, Corcoran GD, Sleator RD *et al*. *Campylobacter corcagiensis* sp. nov., isolated from faeces of captive lion-tailed macaques (*Macaca silenus*). *Int J Syst Evol Microbiol* 2014;64:2878–2883.
38. Gibb S, Strimmer K. MALDIquant: a versatile R package for the analysis of mass spectrometry data. *Bioinformatics* 2012;28:2270–2271.

39. Ahdesmäki M, Strimmer K. Feature selection in omics prediction problems using cat scores and false nondiscovery rate control. *Ann Appl Stat* 2010;4:503–519.
40. Strimmer K. Package 'crossval' Title Generic Functions for Cross Validation 2015.
41. Wheeler NE, Blackmore T, Reynolds AD, Midwinter AC, Marshall J *et al*. Genomic correlates of extraintestinal infection are linked with changes in cell morphology in *Campylobacter jejuni*. *Microb Genom* 2019;5:1–12.
42. Champion OL, Karlyshev AV, Senior NJ, Woodward M, La Ragione R *et al*. Insect infection model for *Campylobacter jejuni* reveals that O-methyl phosphoramidate has insecticidal activity. *J Infect Dis* 2010;201:776–782.
43. Moran AP, Upton ME. Effect of medium supplements, illumination and superoxide dismutase on the production of coccoid forms of *Campylobacter jejuni* ATCC 29428. *J Appl Bacteriol* 1987;62:43–51.
44. Atlas R, Snyder J. Reagents, stains and media: Bacteriology. In: Jorgensen JH, Pfaller MA (editors). *Manual of Clinical Microbiology*. American Society for Microbiology; 2015.
45. MacFaddin JF. *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2000.
46. On SL, Holmes B. Assessment of enzyme detection tests useful in identification of campylobacteria. *J Clin Microbiol* 1992;30:746–749.
47. Ma M, Amano T, Enokimoto M, Yano T, Moe KK *et al*. Influence of pH of TSI medium on the detection of hydrogen sulfide production by *Campylobacter hyointestinalis*. *Lett Appl Microbiol* 2007;44:544–549.
48. Sifré E, Salha BA, Ducournau A, Floch P, Chardon H *et al*. EUCAST recommendations for antimicrobial susceptibility testing applied to the three main *Campylobacter* species isolated in humans. *J Microbiol Methods* 2015;119:206–213.
49. On SL, Holmes B. Reproducibility of tolerance tests that are useful in the identification of campylobacteria. *J Clin Microbiol* 1991;29:1785–1788.
50. Al-Edany AA, Khudor MH, Radhi LY. Isolation, identification and toxigenic aspects of *Campylobacter jejuni* isolated from slaughtered cattle and sheep at Basrah city. *Basrah J Vet Res* 2015;14:316–327.