

## Supplemental materials

Table S1. Primer and probe sequences used in this study.

Purpose	Target		Primer sequence	Detection	Reference <sup>a</sup>	Annealing temperature (°C)	Amplicon Size (bp)
Genus detection	16S	forward	GGATGACACTTTTCGGAGC	Genus	Modified, D Linton[1]	55	800
		reverse 1	CATTGTAGCACGTGTGTC				
	23S	reverse 2	CATTGTAGCACGTGTGTA	Genus	Pholwat, S.[2]	60	158
		forward	GATCCAGTGAAATTGTAGTGGAGGT				
Speciation and phylogenetic analysis	<i>atpA</i>	reverse	GGCTCATATACAACGGCGTCATA	<i>C. jejuni/C. coli</i>	Modified, Dingle[3]	50	1300
		probe	GACGGARAGACCC				
		forward	ATGGACTTAAGAATATTATGGC		Miller[4]	53	924
		reverse 1	ATAAATTCCATCTTCAAATTCC				
		reverse 2	CACGYTCAAGTTGTTTTCTACT(sequence)	<i>C. lari</i>	This study	52	1060
		forward	GARCCAATTGAYGCTAAAGG				
		reverse	TTTAADAVYTCAACCATCTTTGTCC	<i>C. Sp (RM12651)</i>	This study	60	1091
		forward	GGTATGGCHTTAAACCTTGAAGA				
		reverse	TGACCACGCTCWARTTGTCTTCT	<i>C. volucris</i>	This study	60	1091
		forward	GGTGATGCTTTAATTGGTCGTG				
		reverse	AACGGATACAATGCTGCTTCA	<i>C. jejuni</i>	Hill, J. E[5]	60	614
	<i>cpn60</i>	forward	GATCAAGCAGGCGATGGAAC		This study	58	665
		reverse	GCTTTTCTTCTATCGCCAAAACCTG				
		forward	CTTGAAAATATGGGYGCTTCAC	<i>C. coli</i>	This study	56	707
		reverse	GCTTTTCTTCTATCKCCAAAACCT				
		forward	GAAAATATGGGTGCTTCTTTTGT	<i>C. lari</i>	This study	60	651
		reverse	GAAATCACTTCACCACCTGTT				
		forward	CAAGCAGGAGATGGAACACTACAAC	<i>C. Sp (RM12651)</i>	This study	60	655
		reverse	CGTTCCACCTGTAAAGATTGC				
		forward	AAGCWGGTGATGGAACAACAAC	<i>C. ornithocola/ C. subantarcticus</i>	This study	59	680
		reverse	GAAATCACTTCGCCACCTGT				
		forward	CTGATCAAGCAGGAGATGGA	<i>C. volucris</i>	This study	60	122
		reverse	TGTTCTTCCAAGCTCTTCTGAG				
	<i>hipO</i>	forward	CTTGCGGTCATGATGGACATAC	<i>C. jejuni</i>	Liu, J.[6]	60	125
		reverse	AGCACCACCCAAACCCTCTTCA				
	<i>glyA</i>	probe	TGCTTGCTGCAAAGTATT	<i>C. coli</i>	Liu, J.[6]	60	125
		forward	AAACCAAAGCTTATCGTGTGC				
		reverse	AGTGCAGCAATGTGTGCAAT				
		probe	TAAGCTCCAACCTTCATCCG				

Virulence	<i>cdtA</i>	forward	ATCGTACCTCTCCTTGGCG	<i>C. jejuni</i>	Poudel, S.[7]	60	440
		reverse	CGGAGCAGCTTTAACGGTTTG				
		forward	GCCAAGGCTAAAATCTCCAAA	<i>C. coli</i>	This study	60	192
		reverse	TGCCAAGCTCTTGCATCTC				
		forward 1	CGGTTTGGGCTTTAAATCCA	<i>C. lari</i>	This study	58	388
		reverse 1	GCACTAATGCTCCATTGTCTAT				
		forward 2	CAGTTTGGGCCTTAAATCCA				
		reverse 2	GCGCTAACACTCCATTGTCTAT				
	<i>cdtB</i>	forward	TGGGCGATATGTATGATGATG	<i>C. volucris</i>	This study	60	480
		reverse	TCCAAAATTGAGCTTGATTGC				
		forward	TGGAGGAACAGATGTAGGAGC	<i>C. jejuni</i>	Poudel, S.[7]	60	180
		reverse	GCTTGAGTTGCGCTAGTTGG				
		forward	CTAGYAAGTGGGGGAAATGA	<i>C. coli</i>	This study	60	183
		reverse	CGTAGAAGAAGGCGGAACAAC				
		forward	AGAGTDGATGTTGGRGCAAAT	<i>C. lari</i>	This study	60	304
		reverse	TACGCGMTCTTAAATCHGSATCA				
	<i>cdtC</i>	forward	AAAATCACCTGCAACCATCC	<i>C. volucris</i>	This study	60	464
		reverse	CTGCAACCACAGAAAGCAAA				
		forward	GCTCCAAAGGTTCATCTTCTAAG	<i>C. jejuni</i>	This study	60	269
		reverse	GCCTTTGCAACTCCTACTGG				
		forward	GCCTAGCTTGGATGAATTAG	<i>C. coli</i>	This study	60	411
		reverse	TCTATGGCGATACTAGAGTCAG				
		forward	GCAGTTTATAAAYCCTAGAGGTGCTG	<i>C. lari</i>	This study	60	315
		reverse	GGAGTTGCTTCAACRATAGCAG				
	<i>cadF</i>	forward	TCTAAACCGAAACGCTGCTC	<i>C. volucris</i>	This study	60	430
		reverse	GCGTTTGCTGAGATGGAATC				
		forward	CTGCTAAACCATAGAAATAAAATTTCTCAC	<i>C. jejuni/C. coli</i>	Liu, J. [6]	60	221
		reverse	CTTTGAAGGTAATTTAGATATGGATAATCG				
		forward	GCTCAATATGGTGCRGGTTT	<i>C. lari</i>	This study	60	259
		reverse	CACAACCTATATGATCAAGCAAAGC				
		forward	TGGTTATGAGCACCAAAAAGG	<i>C. Sp (RM12651)</i>	This study	60	674
		reverse	TGTCTTCGCTAACACCAGCTT				
	<i>flaA</i>	forward	GTGGTATGTTTGCCCAATATGGT	<i>C. volucris</i>	This study	60	249
		reverse	AGGCACCTTTTCTTGTTCTG				
		forward	ATGCTTCAGGGATGGCGATA	<i>C. jejuni/C. coli</i>	This study	60	178
		reverse	CCATCTTGAGCCGCTTGAGT				
		Forward 2	GATGATGCTTCKGGKATGGCTA	<i>C. lari</i>	This study	59	192
		reverse 2	AGTRCTTTGWCCATCTTGWGC				
		forward	TAACTCTGCTGCTGATGATGCT	<i>C. Sp (RM12651)</i>	This study	60	229
		reverse	AGCTTGAAGTGAGCGTCTTGTT				
	<i>flaA</i>	forward	AGATGATGCTTCAGGTATGGCTA	<i>C. volucris</i>	This study	60	193
		reverse					

	reverse	AGTGCTTTGCCCATCTTGAG				
	forward 1	ATGCTTCAGGTATGGCTATTG	<i>C. volucris</i>	This study	56	418
	reverse 1	CTACTTTTAGCTTGAGATCCTGTT				
<i>cheY</i>	forward	TGATGACAGTTCTACTATGAGAAGG	<i>C. jejuni</i>	This study	56	362
	reverse	CTTCTCCACTTCCTGTTCT				
	forward	TGATGACAGTTCTACTATGAGAAGG	<i>C. coli</i>	This study	56	283
	reverse	CAGTAATCACCTCAGCCTTG				
	forward	GATGTTGATGATAGTTCTACCATGAG	<i>C. lari</i>	This study	56	248
	reverse	CCACCTTCTGTWGTACCATGA				
	forward	CAAAGATTAGGTCATGAGGATG	<i>C. Sp (RM12651)</i>	This study	56	210
	reverse	TTCTGCTTTACCACCTTCAG				
	forward	GATGTTTTAGAGGCTGAGCA	<i>C. volucris</i>	This study	56	198
	reverse	TTCAGCTTTACCACCTTCTG				
<i>CiaB</i>	forward	AGCTGAATTTGCTTGGAATGA	<i>C. jejuni</i>	This study	58	279
	reverse	CCATAAAATATCATCGGAGTGC				
	forward	GGCTGAATTTGCATGGATGA	<i>C. coli</i>	This study	60	306
	reverse	GCAGAAAACAAACCTTAAGCTC				
	forward	GTTCAAGGTGTGCATAATATAGGTGT	<i>C. lari</i>	This study	58	704
	reverse	CCCTTAAGCTCTGTCYCCATAA				
	forward	AGACTTCATTCAAGTTGGGCATT	<i>C. Sp (RM12651)</i>	This study	60	493
	reverse	TTCGTGTCCAATGGTGCTAA				
	forward	AGCTGAACTTGCATGGATGG	<i>C. volucris</i>	This study	60	298
	reverse	AAGTCCTTTAAGCTCTGCTCCA				
<i>gluP</i>	forward	AGCTCCACCCCAATAATAAGC	<i>C. Sp (RM12651)</i>	This study	60	604
	reverse	TCAAAATGGTGTTGTAGGTGGA				
<i>hlyB</i>	forward	TAGTTTGCTCTTGCGGTGTAT	<i>C. Sp (RM12651)</i>	This study	58	695
	reverse	CGCAAAATTAGGCAGTGAGT				
<i>pgiB</i>	forward	TGGATGATGTAAATGCAGTGCT	<i>C. Sp (RM12651)</i>	This study	60	662
	reverse	TGCGATTATATGTGARCCACCA				
<i>gyrA</i> T86I	forward	GCCCGTATAGTGGGTGCTGT	<i>C. jejuni</i>	Pholwat, S.[2]	60	86
	reverse	TCTTGAGCCATTCTAACCAGAGC	fluoroquinolone			
	probe <sup>W</sup>	ATAAACTGCTGTATCTC	resistance			
	probe <sup>M</sup>	AACTGCTATATCTCC				
23S A2075G	forward	GATCCAGTGAAATTGTAGTGGAGGT	<i>Campylobacter</i>	Pholwat, S.[2]	60	158
	reverse	GGCTCATATACAACCTGGCGTCATA	macrolide			
	probe <sup>M</sup>	GACGGAAAGACC	resistance			
	Probe <sup>W</sup>	GACGGAGAGACCC				

<sup>a</sup> The original primers and probes were used as previously published if it was not indicated as “Modified” or “This study”.

## Reference

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Table S2. Distribution of *Campylobacter* detected in different migratory bird species at 4 habitats.

Migratory birds Order	Family	Genus	Hebei	Heilongjiang	Qinghai	Xizang	Total
Anseriformes	Anatidae	<i>Anas platyrhynchos</i>		2			2
		<i>Anser albifrons/fabalis</i>	1	31	1		33
		<i>Anser anser/Anser erythropus</i>			4	1	5
		<i>Anser indicus</i>	13		11	47	71
		<i>Aythya nyroca</i>			1		1
Charadriiformes	Laridae	<i>Chlidonias leucopterus</i>	1				1
		<i>Chroicocephalus ridibundus</i>	16			1	17
		<i>Ichthyaetus relictus</i>	1				1
		<i>Larus argentatus</i>	1				1
	Scolopacidae	<i>Numenius arquata</i>	2				2
		<i>Tringa erythropus</i>				1	1
	Recurvirostridae	<i>Recurvirostra avosetta</i>	2				2
	Charadriidae	<i>Charadrius placidus</i>	1				1
		<i>Pluvialis squatarola</i>	1				1
Suliformes	Phalacrocoracidae	<i>Phalacrocorax carbo</i>	4			1	5
Strigiformes	Strigidae	<i>Athene noctua</i>	1				1
Falconiformes	Falconidae	<i>Falco peregrinus</i>		1			1
Total			44	34	17	51	146

Table S3. Detection of virulence genes in 5 *Campylobacter* species.

Virulence gene	Function	Detection Rate (%)*				
		<i>C. jejuni</i>	<i>C. coli</i>	<i>C. lari</i>	<i>C. volucris</i>	RM12651-like
<i>cdtA</i>	Exotoxin	76.5 (13/17)	66.7 (2/3)	81.8 (9/11)	75.0 (3/4)	-
<i>cdtB</i>	Exotoxin	76.5 (13/17)	66.7 (2/3)	81.8 (9/11)	50.0 (2/4)	-
<i>cdtC</i>	Exotoxin	23.5 (4/17)	33.3 (1/3)	81.8 (9/11)	50.0 (2/4)	-
<i>cadF</i>	Adherence	70.6 (12/17)	33.3 (1/3)	91.7 (11/12)	100.0 (4/4)	84.3 (59/70)
<i>cheY</i>	Motility	86.7 (13/15)	66.7 (2/3)	91.7 (11/12)	100.0 (4/4)	86.3 (63/73)
<i>flaA</i>	Motility	93.3 (14/15)	66.7 (2/3)	100.0 (12/12)	75.0 (3/4)	91.8 (67/73)
<i>ciaB</i>	Invasion	80.0 (12/15)	100.0 (3/3)	83.3 (10/12)	100.0 (4/4)	84.3 (59/70)
<i>gluP</i>	Immune modulation	-	-	-	-	75.7 (53/70)
<i>pgiB</i>	Immune modulation	-	-	-	-	78.1 (57/73)
<i>hlyB</i>	Exotoxin	-	-	-	-	72.6 (53/73)

\*Due to the availability of the samples, no test was done on a few samples. Only samples with complete tests were included for Figure 2 and Figure 3B.

#### Supplemental Figure Legends

Figure S1. Phylogenetic analysis based on 16S rRNA gene in different habitats and migratory birds. Fasttree was used to construct the tree. *Helicobacter* was used as an outgroup. The figure was prepared with iTOL (Interactive Tree of Life). Different colors in the two columns represent different regions and birds, respectively. The Bootstrap value is displayed only when <0.7.

Tree scale: 0.1

