Phenotypic Characters and Molecular Epidemiology of *Campylobacter Jejuni* in East China

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Abstract: In this study, we investigated the distribution, phenotypic and molecular typing characters of *Campylobacter jejuni* in domestic fowl, and livestock populations in East China, to provide some reference for researches on its molecular epidemiology. A total of 1250 samples were collected from different animal sources, and *C. jejuni* strains were then isolated and tested for antibiotic sensitivity. Antibiotics-resistance gene and pathogenic genes were detected by polymerase chain reaction. Phylogenic analysis on the *C. jejuni* strains was performed by multilocus sequence typing (MLST) method. The results showed that 108 out of the 1250 samples (mean 8.64%) were *C. jejuni* positive. These 108 *C. jejuni* strains were highly sensitive to antibiotics such as chloramphenicol, amoxicillin, amikacin, cefotaxime, and azithromycin, whereas they were highly resistant to antibiotics such as cefoperazone, cotrimoxazole, cefamandole, sulfamethoxazole, and cefradine. Pathogenicity related gene identification indicated that the mean carrying rate of adhesion related gene cadF and racR, flagellin gene flaA, toxin regulating gene cdtA, cdtB, cdtC, wlaN and virB11, heat shock proteins and transferring proteins related genes dnaJ and ceuE, CiaB and pldA were 92.45%, 38.69%, 73.58%, 71.70%, 52.83%, 96.23%, 12.26%, 1.89%, 0.94%, 65.09%, 39.62% and 9.43%, respectively. A total of 58.82% of these strains contained more than 6 pathogenicity-related genes. MLST typed 58 ST types from the 108 isolated *C. jejuni* strains, including 24 new types, and ST-21 was the major type, accounting for 39.3% of the total strains.

Keywords: Campylobacter jejuni, multilocus sequence typing (MLST), pathogenic gene

Practical Application: The diversified and complicated situations of epidemiology and drug resistance of *C. jejuni* strains from East China regions were revealed in this study, with the usual occurrence of pathogenicity-related genes and the difference of drug resistance and multiple drug resistance spectrums of strains from different host animals. MLST inspection revealed a fast evolution of *C. jejuni* strains from animal sources in this region with a significant genetic polymorphism. The ST type had no strict relationship with drug resistance or pathogenicity-related genes.

Introduction

Campylobacter is an important zoonotic pathogenic bacterium, causing diarrhea, abortion, human acute enteritis, Guillain–Barre syndrome (GBS), reactive arthritis, Reiter's syndrome, and other diseases. It is widely found in the intestinal tract of the wild and domestic animals and birds as a normal parasitic germ (Park 2002). The outbreak of *Campylobacter jejuni* attracts much attention around the world (Skirrow and Blaser 2000). *C. jejuni* and *Campylobacter coli* can cause diseases in human and animals by direct contact or food contamination. Fowl and dairy cow are the main host of *C. jejuni*, while pig is the main host of *C. coli* (Senok and others 2007). Monitoring the distribution of *Campylobacter* is proposed as the important preventive and control approach. In this study, we regularly collected samples from different animal sources and isolated *C. jejuni* in East China and inspected the antibiotics resistance and related genes and pathogenicity-related genes of these *C. jejuni* strains to provide useful data for controlling the disease.

To determine the evolutionary law of certain bacteria, multilocus sequence typing (MLST) is often used for systematic epidemiological investigations as a useful population genetics tool (Pradeep and others 2008). MLST is first proposed by Dingle in 2001 for the study of the origin and evolution of *C. jejuni* (Dingle and others 2001), and it has been recognized as a valuable tool for epidemiological inspection of *C. jejuni* after verification in large number of experiments (Dingle and others 2005). In this study, based on the researches on antibiotics resistance, resistant genes, and pathogenicity-related genes, we used MLST technology to investigate the phylogeny of 108 *C. jejuni* isolates to reveal the evolutionary relationships between isolates from different regions and animal sources, and provided a reference for similar researches.

Materials and Methods

Bacteria strains

108 bacterial strains were isolated from 1250 swab samples of chicken, duck, goose, dairy cow, pig, monkey and red-crowned crane, and preserved by national key lab for Agricultural product and food Inspection, Jiangsu Entry-Exit Inspection and Quarantine Bureau, China. These swab samples were collected from different animal sources using swabs during May 2006 to October

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doi: 10.1111/1750-3841.13146

Journal of Food Science A Publication of the Institute of Food Technologists

Table 1–Detection of drug resistant genes by PCR.

Gene	Primer sequence	Product (bp)	Annealing Temperature (C°)
gyrA	5′TACGATTTGTITCATTG3′ 5′ATTTCTTTAGCAGGCATA3′	673	45
parC	5′ AAACCTGTTCAGCGCCGCATT3′ 5′ GTGGTGCCGTTAAGCAAA3′	912	55
class 1 integrons int1	5′ GGCATCCAAGCAGCAAGC3′ 5′ AAGCAGACTTGACCTGAT3′	230	55
tet(o)	5′ GGCGTTTTGTTTATGTGCG3′ 5′ ATGGACAACCCGACAGAAGC3′	559	50
CJ1/DP1	5′ TCAAGCTGGTTAGCTA3′ 5′ ACGGCGGCCGTAACTATA3′	300	52
mutation	5′ ATGTCGGCTCATCGCATCCTGG3′ 5′ CATCCATTACACACCCAGCCTATC3′	300	56
CJ18/CJ19	5′ GAAGTTGTATCTTATGATGCTGAAAA3′ 5′ TAACTACGACACCTTGCT CTTG3′	800	60
CJ20/CJ21	5′ TCCGGTTTATATTACTGAA3′ 5′ CTTTAGTTGGAAACCATCTTG3′	600	50
CJ copy-R	5′ CTACCCACCAGACATTGTCCAC3′		52
F1	5′ CCTAAGTCAAGCCTTTCAATCC3′		
F2	5′ CGTTATAGATACGCTTAGCGGTTATG3′		
F3	5′ CATCGAGCAAGAGTTTATGCAAGC3′		

Table 2–Detection of virulence genes by PCR.

Target gene	Sequence (5′→3′)	PCR Product (bp)	PCR reaction condition
Fla A	AATAAAAATGCTGATAAAACAGGTG TACCGAACCAATGTCTGCTCTGATT	855	94 °C 4 min,94 °C 1 min,53 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
cadF	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC	400	94 °C 4 min,94 °C 1 min,45 °C 1 min,72 °C 1 min, 30 cycles, 72 °C 10 min
racR	GATGATCCTGACTTTG TCTCCTATTTTTACCC	584	94 °C 4 min,94 °C 1 min, 45 °C 1 min,72 °C 1 min, 35 cycles, 72 °C 10 min
dn aJ	AAGGCTTTGGCTCATC CTTTTTGTTCATCGTT	720	94 °C 4 min,94 °C 1 min, 46 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
virB11	TCTTGTGAGTTGCCTTACCCCTTTT CCTGCGTGTCCTGTGTTATTTACCC	494	94 °C 4 min,94 °C 1 min, 53 °C 1 min, 72 °C 1 min, 30 cycles, 72 °C 10 min
ciaB	TTTTTATCAGTCCTTA TTTCGGTATCATTAGC	986	94 °C 4 min,94 °C 1 min, 42 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
pldA	AAGCTTATGCGTTTTT TATAAGGCTTTCTCCA	913	94 °C 4 min,94 °C 1 min, 57 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
cdtA	CCTTGTGATGCAAGCAATC ACACTCCATTTGCTTTCTG	370	94 °C 4 min,94 °C 1 min, 57 °C 1 min, 72 °C 1 min, 30 cycles, 72 °C 10 min
cdtB	CAGAAAGCAAATGGAGTGTT AGCTAAAAGCGGTGGAGTAT	620	94 °C 4 min,94 °C 1 min, 57 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
cdtC	CGATGAGTTAAAACAAAAAGATA TTGGCATTATAGAAAATACAGTT	182	94 °C 4 min,94 °C 1 min, 57 °C 40 s, 72 °C 40 s, 30 cycles, 72 °C 10 min
wlaN	TTAAGAGCAAGATATGAAGGTG CCATTTGAATTGATATTTTTG	762	94 °C 4 min,94 °C 1 min, 57 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min
ceuE	CCTGCTACGGTGAAAGTTTTGC GATCTTTTTGTTTTGTGCTGC	793	94 °C 4 min,94 °C 1 min, 57 °C 1 min, 72 °C 1 min, 35 cycles, 72 °C 10 min

2008, and delivered in Carry-Blair transport media to lab within 24 h. After 18 to 24 h of enrichment, the culture was plated on modified Skirrow plate and preserved in microaerophilic containers or at 42 °C of microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2). Observe the culture after 48 h of incubation and identify the bacterial strain according to the literature (Xue and others 2009).

The standard strain of *C. jejuni*, ATCC33560, was purchased from China bacterial strain center, and the control strains of *Escherichia coli* and Salmonella were from our own lab.

Antimicrobial sensitivity test

Germ solutions were brushed on Charcoal Cefoperazone Deoxycholate Agar (CCDA) antimicrobial sensitivity plate and tested according to agar diffusion method recommended by CLSI (Wayne 2005). The CCDA antimicrobial sensitivity plates were checked after 24h of culture at 42 °C for antimicrobial circles according to CLSI/NCCLS standard, with *E. coli* and *Salmonella* strains as comparison.

PCR identification of drug resistant genes

According to literature (Gibreel and others 1998; Fiona and others 2004), primers were designed based on the common 11 drug-resistant genes of *C. jejuni* (Table 1) and synthesized by TaKaRa Co (Da Lian, China). In a typical polymerase chain reaction (PCR) reaction, 25 μL PCR system contained 2.5 μL of 10 × buffer, 1.5 μL dNTPs (deoxy-ribonucleoside triphosphate) (2.5 mM), 1 μL primer each(2.5 μm), 2 μL DNA template, and 0.5 μL Taq polymerase (3 U/μL). The general PCR protocol was 35 cycles of 1 min of predenaturation under 95 °C, 30 s of denaturation under

Table 3–Antibiotic sensitivity of 108 isolated *C. jejuni* strains.

Class	Names of antibiotics	Dose (μg/disc)	Restrain	Moderate sensitivity	High sensitivity	Sensitivity
Penicillin	Penicillin g	10	82.41	7.40	10.19	17.59
	Ampicillin	10	37.96	23.15	38.89	62.04
	Amoxicillin	30	0.93	0.93	98.14	99.07
Cephalosporins	Cefoperazone	30	99.07	0	0.93	0.93
	Cephalexin	30	91.67	1.85	6.48	8.33
	Cefotaxime	30	8.33	25.93	65.74	91.67
	Cefamandole	30	99.07	0	0.93	0.93
	Cefaclor	30	34.26	14.81	50.93	65.74
Aminoglycosides	St reptomycin	10	17.59	3.70	78.71	82.41
	Gentamicin	10	14.81	10.19	75	85.19
	Kanamycin	30	19.45	12.96	67.59	80.55
	Spectinomycin	10	14.81	2.78	82.41	85.19
	Amikacin	30	7.41	3.70	88.89	92.59
Quinolones	Norfloxacin	10	87.04	5.56	7.40	12.96
	Ciprofloxacin	5	85.19	6.48	8.33	14.81
	Levofloxacin	5	69.44	4.63	25.93	30.56
	Nelidixic acid	30	86.11	2.78	11.11	13.89
	Enrofloxacin	5	81.48	2.78	15.74	18.52
Sulfonamides	Co-rimoxazole	25	99.07	0.93	0	0.93
	Sulfamethorxazole	25	97.22	0.93	1.85	2.78
Macrolides	Erythromycin	15	12.96	14.81	72.22	87.03
	Azithromycin	15	9.26	5.56	85.18	90.74
Tetracyclines	Tetracycline	30	75.00	9.26	15.74	25.00
	Oxytetracycline	30	80.56	7.40	12.04	19.44
	Doxycycline	30	78.70	6.48	14.82	21.30
Clindamycin	Clindamycin	2	12.96	9.26	77.78	87.04
Chloromycetins	Chloramphenicol	10	0	5.56	94.44	100

Table 4–Resistance of *C. jejuni* isolates to 27 antibiotics.

	Resistance/rates of multiresistance%*																			
Host	5R	6R	7R	8R	9R	10R	11R	12R	13R	14R	15R	16R	17R	18R	19R	20R	21R	22R	23R	24R
Swine	0	4	0	0	4	12	4	4	4	12	12	12	0	4	4	4	0	8	4	4
Chicken	1.61	0	6.45	1.61	4.84	8.06	1.61	8.06	9.68	14.52	17.74	11.29	8.06	1.61	0	1.61	1.61	0	0	0
Dairy cow	0	25	0	0	0	0	0	0	0	0	25	12.5	0	12.5	12.5	12.5	0	0	0	0
Duck	0	0	0	0	0	11.11	0	0	11.11	0	11.11	55.55	11.11	0	0	0	0	0	0	0
Goose									50.00			50.00								
red-crowned crane						100														
Monkey										50.00		50.00								

*5R-24R represents multiple resistances from 5 to 24 antibiotics.

Table 5–Presence of virulence-associated genes in *C. jejuni* isolates from different species.

	flaA	*cadF*	*racR*	*dnaJ*	*ciaB*	*cdtA*	*VirB*	*pldA*	*cdtB*	*cdtC*	*walN*	*ceuE*
Chicken	83.87	93.55	43.55	0	33.87	82.26	1.61	14.52	50	98.39	14.52	70.97
Duck	100	100	50	0	0	87.5	0	12.5	50	87.5	12.5	100
Pig	36.84	89.47	21.05	0	47.37	52.63	0	0	52.63	94.74	15.79	42.11
Cattle	54.55	90.91	27.27	0	72.73	54.55	9.09	0	72.73	90.91	0	72.73
Goose	100	50	50	0	50	100	0	0	50	100	50	100
Monkey	100	100	50	0	50	0	0	0	50	100	0	50
Crane	100	100	50	0	50	50	0	0	50	100	50	100

95 °C, 1 min of annealing at a varied temperature, and 1 min of elongation under 72 °C, and with a last 10 min of elongation under 72 °C. The PCR products underwent agarose gel electrophoresis (1%), and were observed and photographed on ChemiDoc XRS+ Gel Imaging System (USA, BIO-RAD company).

PCR detection for virulence genes

According to the literature (Hernandez and others 1995; Kaisar and others 2008), primers were designed with the protocols based on the 12 virulence genes (Table 2): flaA, cadF, racR, dnaJ, virB11, ciaB, pldA, cdtA, cdtB, cdtC, wlaN, virB11, dnaJ, and ceuE, and synthesized by Nanjing Jinsirui Co (Nan Jing, China). In a typical PCR reaction, 25 μL PCR system contained 2.5 μL of 10 × buffer, 2 μL dNTPs (10 mM), 2 μL primer each (2.5 μM), 2 μL DNA template, and 0.2 μL Taq polymerase (5 U/μL). The PCR products underwent agarose gel electrophoresis (1%), and were observed and photographed on Gel Imaging System.

MLST typing

The genomic DNA of fresh bacterial liquid will be picked up by the kit (TAKARA). As the target genes, 7 house-keeping genes of *C. jejuni*, including *aspA* (encoding aspartase A), *glnA* (glutamine synthase), *gltA* (citrate synthase), *glyA* (serine

Table 6–MLST typing of the isolates from different sources.

Source	Isolate	Isolation date	Sequence type	Clonal complex
Chicken ($n = 66$)	A110611	June 14, 2006	2845	ST-21 complex
	A110608	June 14, 2006	19	ST-21 complex
	A110604	June 14, 2006	NEW1	NA*
	A110612	June 14, 2006	185	ST-21 complex
	A110603	June 14, 2006	NEW2	NA
	A110601	June 14, 2006	NEW3	NA
	A110602	June 14, 2006	NEW4	NA
	A110605	June 14, 2006	760	ST-21 complex
	A110609	June 14, 2006	2845	ST-21 complex
	A110907	Sept. 2, 2006	1359	ST-21 complex
	A110913	Sept. 2 2006	21	ST-21 complex
	A110901	Sept. 2, 2006	50	ST-21 complex
	A110915	Sept. 2, 2006	1919	ST-52 complex
	A110903	Sept. 2, 2006	NEW5	NA
	A110908	Sept. 2, 2006	NEW6	NA
	A111016	Oct. 11, 2006	NEW7	NA
	A111020	Oct. 11, 2006	21	ST-21 complex
	A111028	Oct. 11, 2006	NEW8	NA
	A111029	Oct. 11, 2006	NEW9	NA
	A111022	Oct. 11 2006	NEW10	NA
	A111021	Oct. 11, 2006	2929	ST-21 complex
	A111025	Oct. 11, 2006	1811	ST-21 complex
	A111027	Oct. 11, 2006	1952	ST-21 complex
	A111023	Oct. 11, 2006	21	ST-21 complex
	A120110	Jan. 10, 2007	4108	ST-607 complex
	A121113	Nov. 15, 2007	2282	ST-206 complex
	A121132	Nov. 15, 2007	577	ST-21 complex
	A121127	Nov. 15, 2007	NEW11	NA
	A121120	Nov. 15, 2007	NEW12	NA
	A121131	Nov. 15, 2007	NEW13	NA
	A121135	Nov. 15, 2007	1811	ST-21 complex
	A121113	Nov. 15, 2007	2282	ST-206 complex
	A121112	Nov. 15, 2007	185	ST-21 complex
	A121133	Nov. 15, 2007	822	ST-21 complex
	A121116	Nov. 15, 2007	NEW14	NA
	A121131	Nov. 15, 2007	2929	ST-21 complex
	A121128	Nov. 15, 2007	1811	ST-21 complex
	A121128	Nov. 15, 2007	19	ST-21 complex
	A121121	Nov. 15, 2007	NEW15	NA
	A121134	Nov. 15, 2007	2328	NA
	A121125	Nov. 15, 2007	2247	NA
	A130524	May 23, 2008	113	ST-460 complex
	A130527	May 23, 2008	1692	NA
	A130530	May 23, 2008	1692	NA
	A130535	May 23, 2008	8	ST-21 complex
	A130523	May 23, 2008	2044	ST-48 complex
	A130506	May 23, 2008	8	ST-21 complex
	A110003	June 4, 2008	1811	ST-21 complex
	A110001	June 4, 2008	577	ST-21 complex
	A110004	June 4, 2008	NEW16	NA
	A110005	June 4, 2008	21	ST-21 complex
	A110002	June 4, 2008	87	ST-21 complex
	A130611	June 28, 2008	NEW17	NA
	A130614	June 28, 2008	NEW18	NA
	A130613	June 28, 2008	2328	NA
	A130608	June 28, 2008	2433	ST-574 complex
	A140001	Nov. 11, 2008	2	ST-45 complex
	A140002	Nov. 11, 2008	2123	ST-362 complex
	A140003	Nov. 11, 2008	2284	ST-52 complex
	A140004	Nov. 11, 2008	2109	ST-45 complex
	A140005	Nov. 11, 2008	86	ST-21 complex
	A140006	Nov. 11, 2008	1790	NA
	A140007	Nov. 11, 2008	386	ST-206 complex
	A140008	Nov. 11, 2008	25	ST-45 complex
	A140009	Nov. 11, 2008	2563	ST-52 complex
	A140010	Nov. 11, 2008	3446	ST-45 complex
Pig ($n = 18$)	Z110304	Mar. 4, 2008	87	ST-21 complex
	Z110301	Mar. 4, 2008	NEW19	NA
	Z110305	Mar 26, 2008	185	ST-21 complex
	Z130555	May 28, 2008	1811	ST-21 complex
	Z130538	May 28, 2008	1811	ST-21 complex
	Z130524	May 28, 2008	2804	NA

(continued)

Table 6–Continued.

Source	Isolate	Isolation date	Sequence type	Clonal complex
	Z130520	May 28, 2008	676	ST-48 complex
	Z130530	May 28, 2008	3811	ST-353 complex
	Z130507	May 28, 2008	3652	ST-22 complex
	Z130510	May 28, 2008	322	ST-48 complex
	Z130521	May 28, 2008	600	ST-52 complex
	Z130502	May 28, 2008	31	ST-21 complex
	Z130509	May 28, 2008	2266	ST-403 complex
	Z130508	May 28, 2008	NEW20	NA
	Z130527	May 28, 2008	NEW21	NA
	Z130532	May 28, 2008	NEW22	NA
	Z130807	Aug. 11, 2008	2845	ST-21 complex
	Z130824	Aug. 11, 2008	NEW23	NA
Duck ($n = 8$)	D121111	Nov. 13, 2007	2328	NA
	D121113	Nov. 13, 2007	1811	ST-21 complex
	D121116	Nov. 13, 2007	1811	ST-21 complex
	D121114	Nov. 13, 2007	299	ST-206 complex
	D121108	Nov. 13, 2007	50	ST-21 complex
	D121115	Nov. 13, 2007	2328	NA
	D121118	Nov. 13, 2007	50	ST-21 complex
	D131034	Oct. 9, 2008	165	ST-21 complex
Goose ($n = 2$)	E131074	Oct. 9, 2008	3378	ST-42 complex
	E131051	Oct. 9, 2008	45	ST-45 complex
Red-crowned crane ($n = 1$)	D130606	June 28, 2008	802	NA
Ox ($n = 4$)	C130401	Apr. 24, 2008	1936	ST-48 complex
	C130402	Apr. 24, 2008	NEW24	NA
	C130408	Apr. 24, 2008	489	ST-21 complex
	C130403	Apr. 24, 2008	474	ST-48 complex
Cow ($n = 7$)	C131013	Oct. 9, 2008	295	ST-45 complex
	C131027	Oct. 9, 2008	49	ST-49 complex
	C131001	Oct. 9, 2008	21	ST-21 complex
	C131038	Oct. 9, 2008	761	ST-21 complex
	C131032	Oct. 9, 2008	2319	ST-403 complex
	C131003	Oct. 9, 2008	42	ST-42 complex
	C131029	Oct. 9, 2008	3378	ST-42 complex
Monkey ($n = 2$)	H130311	Mar. 19, 2008	842	ST-21 complex
	H130312	Mar. 19, 2008	52	ST-52 complex
Human GBS ($n = 1$)	H110001	2006	New	NA

*NA means not applicable.

hydroxymethyltransferase), p*gm* (phosphoglucomutase), *tkt* (transketolase), and *uncA* (ATP synthase alpha subunit), will be adopted to conduct PCR amplification. Primers were designed referring to the MLST website (www.mlst.net) and synthesized by Nanjing Jinsirui Co., China. The PCR protocols were based on the MLST website database. The sequencing of the PCR product was carried out by Nanjing Jinsite Co., China.

Each PCR product was sequenced from both directions, and the sequences were analyzed by BLAST on the GenBank database and conducted Clustal V analysis using Lasergene 7.1. To limit errors, the last base at each end of the reading was excluded. The final sequencing results were compared with the MLST website database using minimum-spanning tree and Tree drawing software.

Results

Distribution of *C. jejuni*

A total of 1250 samples were collected in East China during 2006 to 2008, among which 108 were *C. jejuni* positive (8.64%). For different sources, the positive rate of chicken was 14.08% (66/490), 8% (8/100) from duck, 5% (2/40) from goose, 7.33% (11/150) from dairy cow, 9% (18/200) from pig, 2% (2/100) from monkey, 1.25% (1/80) from red-crowned crane, and no positive case (0/80) from pronghorns (Table 1).

Antibiotics resistance

These 108 *C. jejuni* strains were highly sensitive (more than 80% sensitive) to antibiotics such as chloramphenicol, amoxicillin, amikacin, cefotaxime, azithromycin, clindamycin, erythromycin, gentamicin, streptomycin, spectinomycin, and kanamycin; while they were highly resistant (more than 80% resistant) to antibiotics such as cefoperazone, cotrimoxazole, cefamandole, sulfamethoxazole, cefradine, norfloxacin, ciprofloxacin, nalidixic acid, penicillin g, enrofloxacin, and oxytetracycline (Table 3). The antibiotics sensitivity test gave a hint that the current *C. jejuni* isolates were still sensitive to certain types of antibiotics such as aminoglycosides, macrolides, and chloromycetins, while they were resistant to cephalosporins, quinolones, sulfonamides, and tetracyclines, which required great concern in clinical practice.

These 108 *C. jejuni* strains were sensitive to only 11 out of the 27 antibiotics (40.74 %). For antibiotics resistance, multiple resistances were common with resistance of 9 to 20 antibiotics as a main part among these 108 *C. jejuni* strains (85.19%), among which isolates from pig and dairy cow took a main portion (Table 4).

Identification of drug resistance genes

PCR detection for quinolones resistance related genes parC and gyrA revealed that 96.08% of these 108 C. *jejuni* strains were parC positive, but none was gyrA positive. PCR detection also

revealed that only 9.81% were positive for class 1 integrons gene (int1), 45.1% were positive for tet(o), 15.69% was positive for macrolides resisitance related gene, CJ1/DP1, 20.59% were positive for macrolides resisitance related mutation gene, 80.40% were positive for CJ18/CJ19, 77.45% were positive for F1 gene, 97.06% were positive for F2 gene, and 84.32% were positive for F3 gene.

Identification of virulence genes

Among these 108 *C. jejuni* strains, the mean carrying rates of adhesion-related genes cadF and racR were 92.45% and 38.69%, respectively; for flaA gene, it was 73.58%; for cdtA, cdtB, cdtC, wlaN, and virB11 genes were 71.70%, 52.83%, 96.23%, 12.26%, and 1.89%, respectively; for dnaJ and ceuE were 0.94% and 65.09%, respectively; and for CiaB and pldA were 39.62% and 9.43%, respectively, and totally 58.82% of these strains contained more than 6 virulence genes (Table 5).

MLST typing of *C. jejuni* strains from different sources

MLST typing of these samples for the 7 house-keeping genes was compared with public databases by BLAST. The comparison showed that totally 58 ST types, including 24 novel ST types, were detected (Table 6). Among these ST types, chicken source strains contained 32 traditional and 18 novel ST types, pig source strains contained 18 traditional and 4 novel ST types, duck source strains contained 5 traditional and 1 novel ST types, cattle source strains contained 10 traditional and 1 novel ST types, monkey source strains contained only 2 traditional ST types, and goose source strains also contained only 2 traditional ST types. The unique crane strain belonged to ST802 type.

Using eBURSTv3 software to map these strains, all the 108 strains were grouped into 7 main clusters and 37 individual types. The largest cluster containing 26 ST types (Figure 1) and all the types had a common ancestor, ST21, and this cluster also called CC21 complex. The 2nd cluster contained ST2328, ST2247, STNEW23, and STNEW17, with ST2328 predicted as their ancestor. The 3rd cluster contained ST45, ST25, ST2, and ST2109, with ST45 predicted as their ancestor. The 4th cluster contained ST3378, ST42, and STNEW20, with ST3378 predicted as their ancestor. The 5th cluster contained ST52 and ST2563. The 6th cluster contained ST2319 and ST2266. The 7th cluster contained ST4108 and ST1790.

Phylogenic relationship based on MLST analysis

Phylogenic tree was constructed using Tree drawing software based on NJ method (Figure 2). The evolutionary tree reflected the genotypic distribution of strains from different sources. The main ST types for chicken origin were ST21 and ST1811, to which most novel types were similar. Moreover, almost half ST types had close relationship with ST21. However, for chicken, intensive raising mode had a gathering, while free range had scattering ST types. The ST types of pig origin were different among strains, but most of which had close relationship with ST21. The strains of cattle origin contained 11 traditional and 1 novel ST types, and only ST489, ST21, and ST761 were closely related with each other, indicating a remote relationship among these strains. Most ST types of duck origin were closely related with ST21, but the ST2328 type has a remote relationship among these strains. Two strains of goose origin each had a different type but showed a close relationship to each other. Two strains of monkey origin had different types that were remotely related to each other. The only type of crane origin, ST802, had a remote relationship with types from other birds. All the ST types except these newly discovered types, New3, New7, New2, New4, New19, New20, and New12, had close phylogenic relationships with ST21.

Discussion

C. jejuni is an important zoonotic pathogenic bacterium that causes diarrhea in many countries (OzFoodNet 2006), and WHO

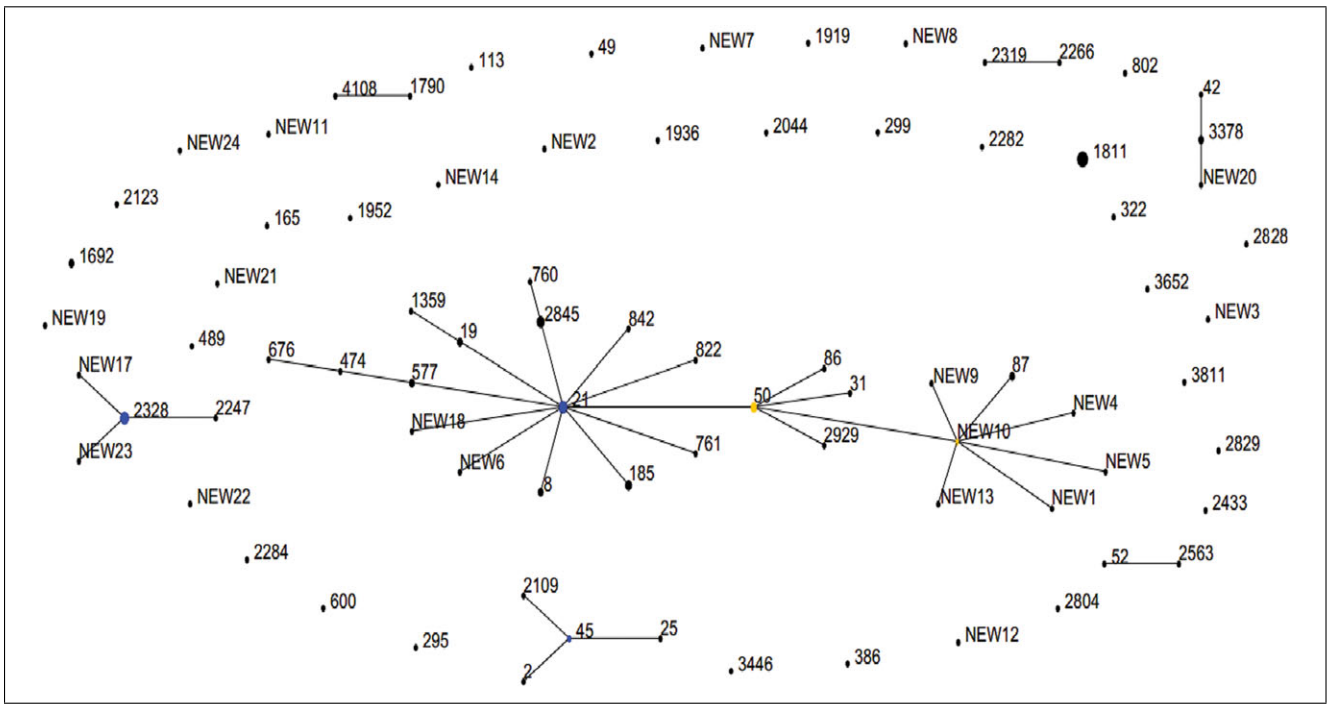

Figure 1–eBURSTv3 diagram for 108 strains of *C. jejuni*. A dot represents ST types, the size of these dots means the relative number of strains, the predicted ancestor of CC group is indicated as blue color, and yellow for subgroup.

lists it as one of the most common foodborne diseases. Campylobacter can colonize on all poultry including chickens, ducks, quails, and ostrich, and wild birds are often infected and colonized (Newell and Wagenaa 2000; Wayne and others 2005). In recent years, the outbreak of Campylobacteriosis raises a major public health concern worldwide, and foreign countries especially developed countries have made risk assessments on the germ from different origins. In this paper, the domestic livestock, fowl, and wild lives were inspected for the carrying rate and drug resistance of *C. jejuni*. The results of separation rate were similar to literatures (Tom and others 2007), with the first report of *C. jejuni* in the red-crowned crane. In general, the infection of *C. jejuni* was complicated and diversified among different animal sources.

The current prevalence of antibiotics resistance in *C. jejuni* raises much concern worldwide (Mead and others 1999; Deepika and others 2005). This worry was also verified in this paper. For the animal sources in this research, they are closely related with human, and people would be infected with the resistant pathogens in contact with these products. A less antibiotics resistance rate was observed in goose, monkey, and crane, which would reflect the less use of antibiotics in practice. The possible drug resistance requires consistent monitoring in free range domestic animals and wild lives.

Detection of the resistance genes also revealed a complicated situation of multiple drug resistance among these isolates, which verified further the drug sensitivity test results. Much attention should be given on the resistance to quinolones and cephalosporins in *C. jejuni*. For success in control of the infection and epidemics of *C. jejuni* with drug resistance, a long-term monitoring system for *C. jejuni* is necessary.

Some researchers have confirmed that some pathogenic genes and regulation mechanism related with pathogenesis of C. *jejuni*. Gene cadF plays an important role in the adhesion and invasion of host cells and widely exists in *C. jejuni* (Konkel and others 1999; Datta and others 2003), which is also verified in this study. Gene racR is reported to play an important role in the pathogenesis of the germ with a high carrying rate (97.79%, Huang and others 2008), which is higher than what present study found (38.69%). The other genes, flaA (Zhang and others 2007) and CDT (cdtA, cdtB, and cdtC), are similar or slightly lower in distribution in this study compared to literature. Otherwise, the interaction of germ and host could be further inspected to determine the relationship between pathogenesis of *C. jejuni* and distribution of pathogenic genes. The mutation in the gene virB11 of plasmid pVir could reduce the adhesion and invasion of the germ (Bacon and others 2000). The general distribution rate of virB11 is reported to be around 10%, while only 1.89% in this study. A total of 58.82% of the isolates containing more than 6 pathogenic genes in this study revealed that the pathogenesis of *C. jejuni* involves a coregulation of multiple pathogenic genes. However, the high carrying rate of pathogenic genes does not mean high pathogenicity, which would be affected by many other factors.

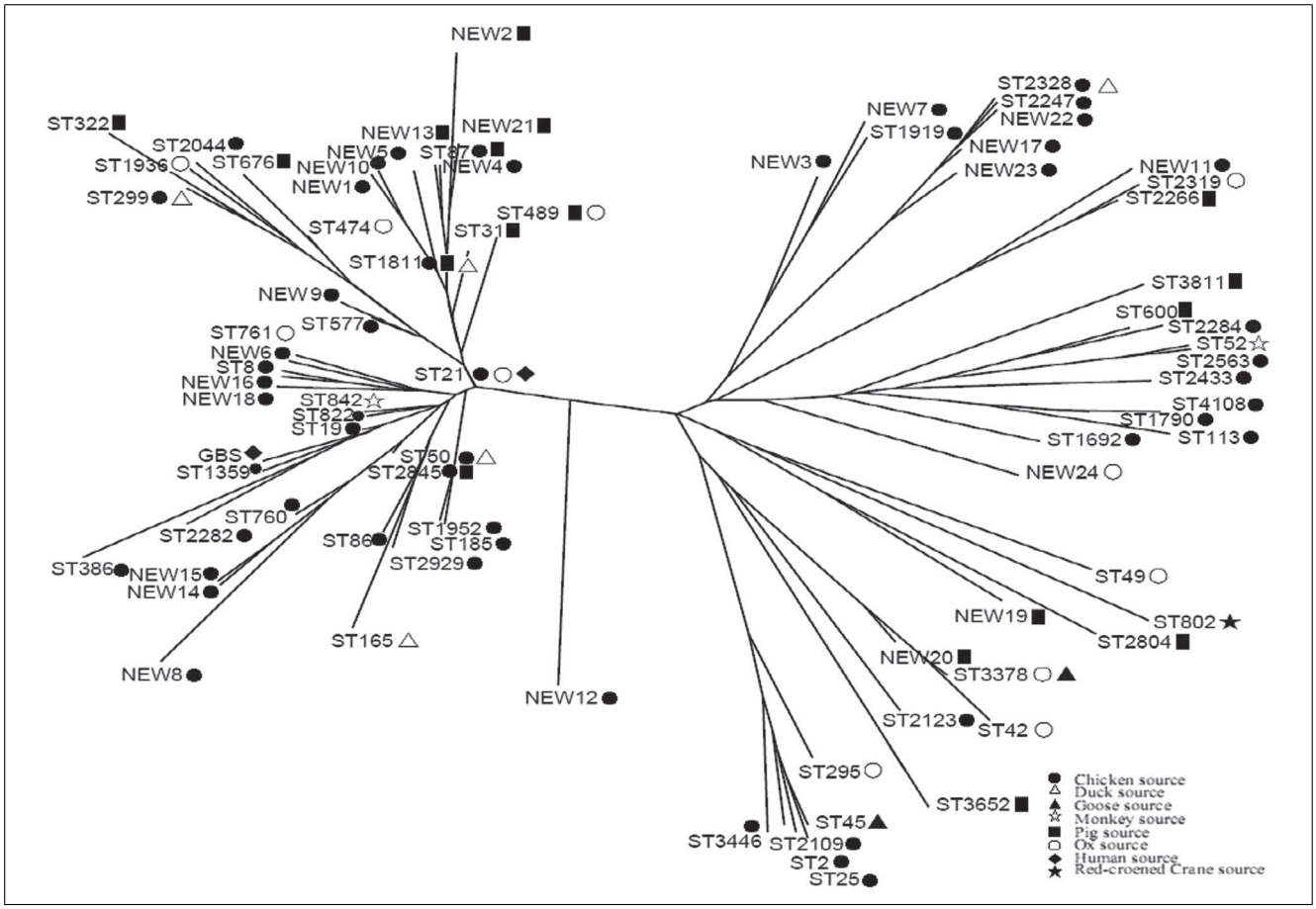

Figure 2–MLST phylogenic tree of *C. jejuni* strains from different sources. The tree was constructed based on NJ method. These novel types were named with NEW.

MLST typing has raised great interest as an accurate molecular typing method for its high resolution, accumulatability, and good repeatability since it was established. Though these strains in this study had different ST types, most strains had close relationship with ST21 according to the phylogenic tree, with the exception of some novel ST types having remote relationship with ST21. Many novel ST types were detected in this study, suggesting that *C. Jejuni* might evolve fast or due to lack of *C. Jejuni* entry of MLST database, especially data from Asian regions.

The main epidemic clone, CC21 complex, revealed in this study by eBURSTv3, is also the popular epidemic clone in China and across the world. ST21 could be the ancestor type based on the international databases and epidemiological resources. In this study, ST50 was proposed to be a subtype of ST21. Five novel types, such as STNEW1, STNEW4, STNEW5, STNEW9, and STNEW13, were the SLV of STNEW10, suggesting that a clone complex (CC) was formed with STNEW10 as its ancestor type. Moreover, ST2328 was expected as the ancestor of a CC, which included ST2247, STNEW17, and STNEW23. The 37 different types, including 11 novel types, had different genetic background and distributed among many animal sources, demonstrating that the infection of *C. jejuni* was complicated and ever-changing.

Combining with the detection results of drug resistance, resistance genes, virulence genes, and MLST typing, it is reasonable to propose that the same ST type can carry different resistance and/or virulence genes, and vice versa. The 3 factors would not be involved with each other.

Conclusions

In this study, the high rate of virulence and multidrug resistance genes in C. *jejuni* gens raised great concern of multidrug resistance. Moreover, the MLST results would complement the MLST database and provide references for epidemiological researches on *C. jejuni*.

Acknowledgments

This research was funded by 863 project 2012AA101601, NSF31301460, Natl 10000 Talents—Youth Top-notch Talent Support Program, The National Science and Technology Support Program 2012BAK17B10, and State Quality Inspection Administration of scientific research project 201510025, 2014IK103, and 2013IK164; We thank Dr. Ji Dejun for the language editing work of this paper.

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