High prevalence of *Campylobacter jejuni* CC21 and CC257 clonal complexes in children with gastroenteritis in Iran: A global analysis

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Abstract

Aims: The aim of this study was to assess the distribution of GBS-related lipo-oligosaccharide (LOS) classes and capsular genotypes among Iranian clinical *C. jejuni* strains and to assess its relation with *dnaK* gene expression. Moreover, a comprehensive study of *C. jejuni* MLST-genotypes and global comparison with peer sequences worldwide was intended.

Methods: Distribution of sialylated-LOS classes and specific capsular genotypes were investigated in *C.jejuni* of clinical origin. The expression of *dnaK* in *C. jejuni* strains was measured by Real-Time-PCR. MLST-genotyping was performed to investigate the clonal relationship of clinical *C. jejuni* strains and comparison with global sequences worldwide.

Results: *C. jejuni* HS23/36c was the predominant genotype (45%), followed by HS2 (20%), and HS19 and HS4 (each 10%). A total of 80% of isolates were assigned to LOS class B and C. Higher expression level of *dnaK* gene was detected in strains with HS23/36c, HS2 and HS4 capsular genotypes and sialylated LOS classes B or C in this study. MLST analysis showed that isolates were highly diverse and represented 6 different sequence types and 3 clonal complexes. ST-21 and ST-257 complexes were dominants (75%) in our *C.jejuni* strains. The CC21 was the largest CC in our collection which is consistent with global *C.jejuni* strains worldwide. No new ST and no common ST with our neighbor countries was detected in this study.

Conclusion: Global analysis of MLST results demonstrated that ST-50 (CC21) was widely distributed in different countries, while ST-19 (CC21) and ST-257 (CC257) was less ubiquitously spread. Overall, CC21 and CC353 complexes were most frequent and most widely distributed clonal complexes around the world; although, CC353 was not detected in this study. This shows a picture of movement of dominant Campylobacter strains worldwide. The occurrence of identical clonal complexes with different capsular types and LOS classes in this study is consistent with genetic variation in circulating identical genotypes and their evolution toward different pathotypes probably through acquisition of different genetic elements including LOS and CPS gene clusters.

Introduction

Campylobacter jejuni (C. jejuni) is a leading cause of bacterial foodborne poisoning and acute gastroenteritis in human worldwide [1]. This bacterium often causes a moderate to severe watery regularly self-limiting diarrhea and post-infectious immune disorders such as Guillain-Barre syndrome (GBS) [2].

Campylobacter jejuni (*C. jejuni*) produces Capsular Polysaccharide (CPS). The CPS gene cluster is located in a hypervariable region in the *C. jejuni* genome. Diversity in the content and gene function together with the presence of phase variable genes in the capsule locus allows the production of broad repertoire of capsular structures [2, 3]. Major serodeterminant of the CPS is in classical Penner or Heat-Stable (HS) serotyping Scheme. In the classical Penner serotyping scheme, which is based on the passive slide hemagglutination assay, *C. jejuni* strains are divided into 47 serotypes, of which, due to similarity in the CPS structure, 35 serotypes have been refined, which are serotype cross-reactive pairs or complexes [4, 5]. *C. jejuni* Penner serotypes associated with Guillain-Barre syndrome (GBS) often belong to HS1, HS4c, HS19, HS23/36c and HS41, furthermore, the most common serotypes among sporadic cases are reported in the HS4c, HS2 and HS1 [4]. Recently, CPS genotyping was used as a more effective method, since it is not sensitive to variations in capsule gene expression either influenced by genes or gene products outside the capsule locus [2], and also it can rapidly and readily determine CPS types in *C. jejuni* [2, 6].

The cluster of genes involved in *C. jejuni* Lipo-Oligosaccharide (LOS) biosynthesis, is one of the most variable regions of *C. jejuni* genome [7]. Among 19 different LOS locus classes from A to S, 3 classes (A, B and C) play a key role in the biosynthesis of the sialic acid and are often isolated from the stools of patients with GBS [2, 7, 8].

Post-infection diseases like GBS with *C. jejuni* have been proved to be associated with antibodies of human gangliosides. The induction of these autoantibodies is associated with molecular mimicry between human gangliosides and bacterial

epitopes present at the surface of the Lipo-Oligosaccharide (LOS) [9] and the antibodies response to diarrhea is different to GBS infected by *C. jejuni* [10]. In addition to antiganglioside antibodies, Heat Shock Proteins (HSP) family can mediate in the autoimmune diseases. They belong to a highly protected family that is present in normal physiological conditions in prokaryotic and eukaryotic cells. These proteins are etiologic factors in many autoimmune diseases in such a way that their overexpression leads to environmental stress induction [9, 11].

C. jejuni carry several of HSPs, including *groELS*, *dnaJ*, *dnaK* and *lon* [12] among which DnaK proteins (70 kDa) has a high homology in its sequence with HSP70 of human peripheral neurons [9]. A high titer of anti-HSP antibody (HSP27, HSP60 and HSP70) can be found in CSF (Cerebrospinal fluid) of patients with GBS [9].

To date, no study has been reported on the distribution of CPS genotypes, LOS locus classes and Multilocus sequence typing (MLST) of *C. jejuni* in Iran. The aim of this study was to assess, for the first time, the distribution of the most commonly found GBS-related LOS classes and capsular genotypes among clinical *C. jejuni* strains isolated from Iranian children. Moreover, the correlation of DnaK protein expression level in *C. jejuni* strains with selected capsular genotypes and LOS classes associated with GBS was intended. Furthermore, a comprehensive comparison of *C. jejuni* MLST genotypes with globally reported peer sequences worldwide was envisioned.

Materials And Methods

Phenotypic and genotypic identification of C. jejuni strains from fecal samples

Two-hundred and eighty fecal specimens collected from children with diarrhea aged 0–5 years referred to 3 Children's Medical Center and Hospitals at Tehran, Iran, from May to October 2018. Information on age, clinical symptoms, history of non-pasteurized dairy products consumption, animal contact as well as laboratory results were recorded. A total of 280 suspected cases of sporadic Campylobacteriosis without history of antibiotic intake were selected from approximately 3000 diarrheal cases. Diarrhea was defined as ≥ 3 episodes per day, accompanied with WBC (white blood cell) and RBC (red blood cell) shedding in a majority of cases. Specimens were transferred to the laboratory using Carry-Blair Transport Media (Micro Media-Hungary) and immediately streaked on Brucella agar and modified charcoal-cefoperazone-deoxycholate agar (mCCDA) (Merck-Germany). Plates were incubated at 42° C for 48 hours under microaerophilic condition using Gas Pack C (Merck-Germany). Gram staining, spiral morphology, catalase and oxidase production, nitrate reduction and indoxyl acetate hydrolysis test were used to confirm *C. jejuni* colonies. Also, hippurate hydrolysis test was used for phenotypic distinguishing of *C. jejuni* from *C. coli*. Finally, twenty *C. jejuni* and three *C. coli* strains were confirmed by Duplex PCR [13].

Identification Of Capsular Genotypes And Los Locus Classes

Since the *C. jejuni* Penner serotypes associated with Guillain-Barre syndrome (GBS) often belong to HS1, HS4c, HS19, HS23/36c and HS41, furthermore, the most common serotypes among sporadic cases are reported in the HS4c, HS2 and HS1 cases [4], the capsular genotypes including HS1, HS2, HS4, HS19, HS23/36c and HS41, PCR were identified in this study according to standard protocols by specific primers (Table 1) [5].

Table 1
The primer sequences included in the *C. jejuni* capsule typing scheme

Primer	Forward sequence	Reverse sequence	Product size (bp)	Reference
HS1	GCAAGAGAAACATCTCGCCTA	TTGGCGGTAAGTTTTTGAAGA	610	[5]
HS2	CATCCTAGCACAACTCACTTCA	CAGCATTGGAGGATTTACAATATAT	62	[5]
HS4A	CCTAACATATCATACACTACGGT	TATATTTGGTTAGGGATCCA	370	[5]
HS19	GGCAACAAACAAACATATTCAGA	CGAGGATGAAAATGCCTCAA	450	[5]
HS23/36	GCTTTATATCTATCCAGTCCATTATCA	GCTTGGGAGATGAATTTACCTTTA	161	[5]
HS41	TGCAATCTCTAAAGCCCAAG	CTTACATATGCTGGTAGAGATGATATG	279	[5]

From 19 different LOS locus classes from A to S, 3 classes (A, B and C) play a key role in the biosynthesis of the sialic acid and are often isolated from the stools of patients with GBS [2, 7, 8]. Therefore, specific set of primers (Table 2) were used for class A, B, and C based on the DNA sequences of specific genes related to LOS classes [7].

Table 2
Primer used for identification of classes A, B and C

ORF	Forward sequence	Reverse sequence	Product size (bp)	Reference
Orf7ab	ACTACACTTTAAAACATTTAATCC AAAATCA	CCATAAGCCTCACTAGAAGGTATGAGTATA	580	[7]
Orf 6ab1	CAAGGGCAATAGAAAGCTGTATCA	ACAAGCACTTCATTCTTAGTATTACAAAT	631	[7]
Orf6ab2	TCATCTTGCCAACTTATAATGTGGA	TCTAGCGATATTAAACCAACAGCCT	517	[7]
Orf5bII	CTGTGATGATGGGAGTGAAGAGC	GGTAATCGTTTCGGCGGTATT	539	[7]
Orf6c	GTAGTAGATGATTGTGGTAATGATAAA	ATAGAATTGCTATTTACATGCTGG	554	[7]
Orf7c	TTGAAGATAGATATTTTGTGGGTAAA	CTTTAAGTAGTGTTTTATGTCACTTGG	746	[7]

PCRs was carried out within a thermal cycler (Eppendorf, Germany) in a final volume of 25 µL containing 1–10 ng DNA template, 2.5 µL 10X PCR buffer, 1 unit of Taq DNA polymerase, 2.0 mM $MgCl_2$, 0.2 µM of each primer, 0.3 mM each dNTP and sterile deionized water. Amplification conditions was as follow: 95 °C for 5 min, followed by 30 amplification cycles; denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min. Finally, an additional extension step (5 min, 72 °C) was performed. Finally, amplicons were electrophoresed on 1% agarose gel.

Real-Time PCR for dnaK **gene expression in clinical** C. jejuni **isolates**

Determination Of Real-time Pcr Primer Efficiency

In order to assess the efficiency of real-time PCR amplification, five serial template dilution of 1:10 served as DNA template for qRT-PCR reaction of the *dnaK and 16srRNA* genes. The CTs values and the concentrations of the template were used to plot the standard curve. In the next step, based on the slope of the standard curve, the primer efficiency was measured. The main reason for calculating the efficiency of primers is to accurately calculate the fold change, which is the output of qRT-PCR reaction [14].

Real-Time PCR for dnaK **gene expression**

RNA extraction was performed on pure cultures of 20 *C. jejuni* strains, which were previously checked for the presence of capsular types and LOS locus classes using a Favoren Biotec Corp kit (Taiwan). Subsequently, the RNA molecules were treated using the DNase I kit (TaKaRa). A cDNA synthesis kit (Yekta Tajhiz Azma-Iran) was used to generate a single-strand cDNA. The cDNAs were kept at -20 °C. Quantitative Real Time-PCR was performed using SYBR Green (RealQ Plus Master Mix Green-Denmark) in Qiagen-Rotor-Gene Q with HRM. 16S rRNA gene was used as the internal control. One of the isolates that neither had the selected capsular serotypes nor the LOS locus classes was considered as a reference gene. The PCR reaction mixture consisted of 100 ng to1 mg of cDNA (for *dnaK* and 16S rRNA genes), 1 mM of each primers (Table 3) [15] and 12.5 mL of SYBR Green I Master Mix. Cycling conditions included an initial denaturing step of 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. $2^{-\Delta\Delta}Ct$ is a relative quantification methods for analyze the relative changes in gene expression from real-time quantitative PCR [15]. SPSS software version 20 was used for the analysis of data.

Table 3
Specific primers used for Real-Time PCR

Primer	Forward sequence	Reverse sequence	Product size (bp)	Reference
dnaK	AAACGCCAAGCGGTAACTAA	TTCTTTAGCCGCGTCTTCAT	90	[15]
16SrRNA	AAGGGCCATGATGACTTGACG	AGCGCAACCCACGTATTTAG	107	[15]

Multilocus Sequence Typing (mlst)

The MLST method relies on the sequence analysis of seven housekeeping genes. The MLST method was performed according to Dingle, et al. [16]. Each 25 µL amplification reaction mixture comprised 1–10 ng DNA template, 1X PCR buffer, 1.25 unit of Taq DNA polymerase, 1.5 mM $MgCl_2$, 1 µM of each primers (Table 4), 0.8 mM each dNTP and sterile deionized water.

Table 4
Primers used for *Campylobacter jejuni* MLST

Primer	Forward	Reverse	Product size (bp)	Reference
asp	CCAACTGCAAGATGCTGTACC	TTAATTTGCGGTAATACCATC	625	[16]
gln	CATGCAATCAATGAAGAAAC	TTCCATAAGCTCATATGAAC	722	[16]
glt	GTGGCTATCCTATAGAGTGGC	CCAAAGCGCACCAATACCTG	575	[16]
gly	AGCTAATCAAGGTGTTTATGCGG	AGGTGATTATCCGTTCCATCGC	648	[16]
pgm	GGTTTTAGATGTGGCTCATG	TCCAGAATAGCGAAATAAGG	700	[16]
tkt	GCTTAGCAGATATTTTAAGTG	ACTTCTTCACCCAAAGGTGCG	691	[16]
unc	TGTTGCAATTGGTCAAAAGC	TGCCTCATCTAAATCACTAGC	631	[16]

The PCR conditions were as follows: denaturation at 94 °C for 2 min; annealing at 50 °C for 1 min, extension at 72 °C for 1 min for 35 cycles. Allelic numbers were identified for each of the housekeeping genes, Sequence Types (ST), and Clonal Complexes (CC) with submitted DNA Sequences in the *C. jejuni* MLST database (http://pubmlst.org/campylobacter) [17]. Dendrogram was plotted using Interactive Tree Of Life (iTOL) v4 [18].

Geographic Distribution Of Sts And Ccs

A phylogenetic analysis revealed that there was no common STs or CCs between Iran and its neighbor countries, Turkey and Pakistan (Fig. 7). This may be due to scarcity of published data from these countries. No data is available from other neighboring countries of Iran. World map related to *C. jejuni* strains from 1980 to 2018 shows the distribution of ST19

(CC21), ST50 (CC21), and ST257 (CC257) among different continents (Figs. 8–10). The frequency of CC21 strains was associated to sporadic cases of human gastroenteritis which was recorded from America (USA and Canada), Europe (UK, The Netherlands and Germany), Asia (Iran, this study) and Australia. Global analysis of CC257 (including ST-257) strains in the same time period showed recorded strains from Asia (Iran, this study), Europe (UK and Spain), America (Chile) and Africa (South Africa).

In a dendrogram constructed by global analysis of MLST results during 2014–2018 a similar picture was depicted for distribution of CC21 and CC257 clonal complexes. Moreover, neighbor-joining results indicated that CC21 and CC353 complexes are the most divers, most frequent and most widely distributed clonal complexes around the world, respectively; although, CC353 was not detected in this study (Fig. 11) (Table 6).

Table 6
Top 10 Clonal complexes based on frequency and diversity extracted from circle dendrogram

Rank	Clonal Complex	Diversity	Rank	Clonal Complex	Frequency
1	ST-353 Complex	12	1	ST-21 Complex	51
2	ST-21 Complex	10	2	ST-353 Complex	42
3	ST-206 Complex	7	3	ST-206 Complex	17
4	ST-48 Complex	6	4	ST-45 Complex	17
5	ST-464 Complex	5	5	ST-48 Complex	14
6	ST-52 Complex	5	6	ST-354 Complex	13
7	ST-574 Complex	5	7	ST-464 Complex	13
8	ST-607 Complex	5	8	ST-403 Complex	10
9	ST-354 Complex	4	9	ST-443 Complex	10
10	ST-1034 Complex	3	10	ST-52 Complex	9

Results

In this study, 23 Campylobacter isolates were identified from 280 stool samples of children with diarrhea. Among the 23 isolates, 20 and 3 isolates were *C. jejuni* and *C. coli*, respectively based on hippurate hydrolysis test and duplex PCR assay of *cadF* gene. The 20 of 23 isolates were capable to hydrolyze hippurate and identified as *C. jejuni*. Analysis of the duplex PCR assay of *cadF* gene showed that 737 and 461 bp amplicons were corresponding to *C. jejuni* and *C. coli*, respectively.

Cps Genotype And Los Locus Class Diversity

From 20 *C. jejuni* isolates, 17 (85%) expressed one of the selected CPS genotypes under study. CPS types HS23/36c were found in 9 isolates (45%), HS2 in 4 (20%), HS19 in 2 (10%) and HS4 in 2 (10%) isolates. The dominant CPS type was HS23/36c.

Of 20 strains 16 (80%) expressed LOS locus class B and C. Class B was dominant in 11 (55%) and class C in 5 (25%) isolates. We found no instance of class A in our collection of isolates.

Relationship Between Cps Type And Los Locus Class

Among *C. jejuni* strains with LOS B class, the dominant CPS types were HS23/36c (n = 6, 54/54%), HS2 (n = 2, 18/18%), HS4 (n = 2, 18/18%) and HS19 (n = 1, 9/9%). Also, the most common CPS genotypes with LOS C class were HS23/36c and HS2

(n = 2, 40%, n = 2, 40%, respectively) (Fig. 1).

Comparison of dnaK gene expression in clinical C. jejuni strains

The + 3.3 ± 10% slope is a reflection of 100%±10% efficiency of real time PCR. Accordingly, the slope was − 3.38 and 3.24 and the efficiency was 97.51% and 103.54 for *dnaK* and 16srRNA genes, respectively. The *dnaK* gene expression was determined by Quantitative Real-Time PCR and according to the $2^{-\Delta\Delta CT)}$ method. Among isolates that showed one of the LOS classes of A-C or one of six selected capsular genotypes, 18 isolates were classified into three groups including group 1: with identified CPS genotype and sialylated LOS class (B or C) (n = 15), group 2: with identified CPS genotype and without sialylated LOS class (n = 2), group 3: without CPS genotype but with sialylated LOS class (n = 1), and one of the isolates that neither had the selected capsular serotypes nor the LOS locus classes was considered as a reference strain in this study. Due to insufficiency of data, differential expression analysis could not be performed; thus, descriptive statistics was used instead. As a result, *dnaK* expression level in group 1 was greater than other groups (Table 5). The *dnaK* expression level was much higher in clinical *C. jejuni* isolates with one of the CPS genotypes and the LOS classes relevant to GBS patients. Based on the $2^{-\Delta\Delta CT)}$ method, the graph of the fold change of *dnaK* gene expression was plotted (Fig. 2).

Table 5
Relationship between the level of *dnaK* gene expression in clinical *C. jejuni* isolates

Group	Isolate ID	Capsular genotype	LOS class	Fold Change: $2^{-\Delta\Delta CT}$
G1	79434	HS2	B	2.04 ± 0.72
G1	79425	HS23/36	B	1.54 ± 0.44
G1	79426	HS23/36	B	1.34 ± 0.3
G1	79438	HS23/36	B	5.43 ± 1.7
G1	79420	HS2	C	2.66 ± 0.99
G1	79437	HS23/36	B	3.0 ± 1.1
G1	79427	HS4	B	2.16 ± 0.78
G1	79435	HS2	B	2 ± 0.7
G1	79439	HS23/36	B	1.5 ± 0.41
G1	79444	HS23/36	C	1.6 ± 0.49
G1	79443	HS4	B	1.70 ± 0.53
G1	79253	HS23/36	C	1.02 ± 0.02
G1	79429	HS19	B	1.34 ± 0.29
G1	79440	HS23/36	B	3.68 ± 1.3
G1	79421	HS2	C	2.65 ± 0.99
				Total: Mean of means: 2.2 ± 0.71
G2	79441	HS23/36	-	1.1 ± 0.12
G2	79428	HS19	-	1.21 ± 0.19
				Total: Mean of means: 1.15 ± 0.15
G3	79442	-	C	1.64 ± 0.45
Reference	79436	-	-	1 ± 0
G1: Isolates with capsular genotype and LOS class				
G2: Isolates with capsular genotype and without LOS class				
G3: Isolates without capsular genotype and with LOS class				

Cc And St Variation Of Mlst Analysis

Based on the MLST analysis of 20 isolates 6 sequence types and 3 clonal complexes were detected in Iran. Five and 7 isolates were identified as sequence type 257 and 50, respectively. Both of the sequence types 19 and 5326 were detected in three isolates. Each of the two sequences type 1096 and 1113 was present in one isolate.

Out of the 3 identified clonal complexes, ST-21 clonal complex dominated in 10 isolates (50%).

Both ST-257 complex and ST-828 complex were found in 5 (25%) and 2 (10%) isolates, respectively.

Distribution of the sequence types and the genetic link between *C. jejuni* strains isolated from patients with diarrhea has been shown in the dendrogram (Fig. 3). The relationship among 20 isolates based on clonal complexes is reflected in minimum spanning tree diagram (Fig. 4) [19].

Linkage between CPS genotypes and LOS class with MLST CC

Totally, 3 CCs (CC828, CC257 and CC21) were identified. The isolates with HS23/36c CPS and HS19 CPS genotype, were found in CC21 (Fig. 5). Majority of isolates in CC21 had LOS class B or LOS class C. In CC257, 4 isolates belonged to HS2 serotype. In this CC, LOS class C and B were observed. Two isolates were assigned to CC828 (Fig. 6).

Discussion

Epidemiological investigations have shown that a *C. jejuni* infection precedes GBS in 20 to 50% of cases in Europe, North and South America, Japan, and Australia [20]. Sialylated LOS loci of A, B and C classes as well as HS types of HS2, HS4, HS23/36c and HS19 are accused to be associated with GBS patients [2, 20, 21]. In the present study we aimed to investigate CPS types and LOS locus classes of virulent *C. jejuni* strains isolated from children in Iran. Among 20 isolates, the prevalent LOS class was B (11/20; 55%), followed by class C (5/20; 25%), while class A was not detected in our collection. Dominant CPS genotype was HS23/36c (9/20) followed by HS2 (4/20). The HS23/36c was not dominant in any of the reported studies and at the global picture, HS4 is the most prevalent type [22], though it was ranked no. 3 in Iran. Similar to our study, HS2 has been reported as the second most prevalent CPS type worldwide [22], while some reports rank it no. 6 in Asia and Africa and no. 7 in some developing countries [22].

A wide range of CPS types including HS1/44, HS2, HS4, HS19 and HS23/36c are usually identified in *C. jejuni* strains isolated from GBS patients [2, 21]. In our enteritis-related samples 85% of strains expressed one of HS23/36c, HS2, HS4 and HS19, which signifies the high probability of GBS progress in this group of patients.

Our findings showed that the *dnaK* expression mean in strains with specified capsular genotype and sialylated LOS was greater than that of others (with either capsular genotype or LOS class). This finding reveals that the expression of *dnaK* was higher in strains when sialylated LOS with particular capsular genotypes simultaneously are present in a strain. Moreover, it was shown by HU et al., that *dnaK* gene expression is upregulated under in-vivo-like conditions which means it may be induced in infected human host. Considering the crucial role of *dnaK* in antigenic mimicry and GBS, it can be concluded that individuals infected with *C. jejuni* strains that have sialylated LOS classes and the selected capsular serotypes as well as a high expression profile of *dnaK* may be more likely to develop GBS. Furthermore, *dnaK* gene can be mentioned as an antigen candidate in preventive studies or as a diagnostic marker [10].

In this study, genetic variations in 20 *C. jejuni* strains was also identified by MLST. A total of 6 STs were observed and 17/20 (85%) belonged to 3 clonal complexes (CCs), while 3 isolates belonged to STs unassigned to a CC.

The majority of *C. jejuni* strains were assigned to CC21; this finding supports previous observations which shows CC21 is the most prevalent CC worldwide. Within CC21, ST-50 was the dominant ST in our clinical samples, although not all of previous studies reported ST-50 as the dominant sequence type [23–27].

Meanwhile, consistent with our finding, ST-50 and ST-19 (CC21) and ST-257 were mostly related to human campylobacteriosis cases [26, 28]. However, isolates from other sources (fresh whole retail chicken, raw milk and environmental water) also presented CC21 as dominant CCs [29].

The correlation between certain MLST clonal complexes and LOS classes and HS types were investigated in present study. The majority of *C. jejuni* isolates in ST-21 (7/10; 70%) expressed LOS class B, while both LOS class B and C occurred in ST-257 complex in an equal percentage (2/5; 40%). Habib et al., demonstrated that ST-21 complex strongly correlated with LOS class C [20] but this combination was rare in our collection.

MLST analysis demonstrated that ST-21 and ST-257 complexes were dominants in our enteritis *C. jejuni* strains. Overall of different studies, the ST-22 complex was significantly overrepresented in the GBS isolates and ST-21 complex in enteritis isolates [2, 21, 25]. No new sequence type was detected in this study. Moreover, among neighbor countries, only a few data from Pakistan and Turkey was available and phylogenetic analysis revealed no common ST or CC with Iran.

Global analysis of MLST results demonstrated that ST-50 (CC21) was widely distributed in different countries including UK, USA, Canada, some European countries, Australia and China, while ST-19 (CC21) and ST-257 (CC257) was less ubiquitously spread and absent from Australia and China/Canada, respectively.

Moreover, neighbor-joining results indicated that CC21 and CC353 are the most diverse, most frequent and most widely distributed clonal complex around the world; although, CC353 was not detected in this study. The most diverse CCs are related to more prevalent sequence types. This proposes that probably their diversity is a mirror of their replication frequency and circulation which affects their gene content and efficiency. This shows the movement of Campylobacter strains beyond the boundaries. The occurrence of identical clonal complexes with different capsular types and LOS classes in this study is consistent with genetic variation in circulating identical genotypes and their evolution toward different pathotypes probably through acquisition of different genetic elements including LOS and CPS gene clusters.

Conclusions

For the first time this study presents MLST typing as well as identifying CPS types and sialylated LOS classes of clinical *C. jejuni* strains isolated from Tehran, Iran.

In conclusion, the present study demonstrated that *C. jejuni* isolates had the predominant LOS class (B and C) and capsular genotypes (HS23/36, HS2, HS4 and HS19) which are supposed to be related to GBS; Isolates from this study were highly genetically diverse in 6 STs. ST-21 and ST-257 clonal complexes were predominant in our collection. ST-21 complex is also the largest clonal complex in global *C. jejuni* strains. No new ST and no common ST with our neighbor countries was detected in this study.

Global analysis of MLST results demonstrated that ST-50 (CC21) was widely distributed in different countries, while ST-19 (CC21) and ST-257 (CC257) was less ubiquitously spread. This study shows a picture of movement of Campylobacter strains around the world. The occurrence of identical clonal complexes with different capsular types and LOS classes in this study is consistent with genetic variation in circulating identical genotypes and their evolution toward different pathotypes probably through acquisition of different genetic elements including LOS and CPS gene clusters.

Abbreviations

C. jejuni: Campylobacter jejuni; CPS: Capsular Polysaccharide; HS: Heat-Stable; GBS: Guillain-Barre syndrome; LOS: Lipo-Oligosaccharide; HSP: Heat Shock Proteins; MLST: Multilocus sequence typing; mCCDA: modified charcoal-cefoperazone-deoxycholate agar; ST: Sequence Types; CC: Clonal Complexes; iTOL: Interactive Tree of Life

Declarations

Author Contributions

Mahnaz Sarhangi collected all specimens and performed all the laboratory tests, also had a major contribution in drafting the main manuscript and prepared all figures and tables and had approved the submitted version.

Bita Bakhshi designed and supervised the study entirely and had a major contribution in writing the manuscript and had approved the submitted version.

Shahin Najar Peeraeyeh had has a major role in analysis of the data as well as drafting the main manuscript and had approved the submitted version.

Ethics approval and consent to participate

The study was reviewed and approved by the Medical Ethics Committee of Tarbiat Modares University (Code: IR.MODARES.REC) before it began and all research was performed in accordance with relevant guidelines/regulations. The clinical specimens were obtained from Microbiology Laboratory of 3 children hospitals, Tehran, Iran. The consent to participate was obtained from the parents/guardians of the minors included in this study and the data were analyzed anonymously.

Consent for publication

Not applicable.

Availability of data and materials

The datasets of the current study are available within article or can be obtained from corresponding upon request. DNA sequences of genes that have been deposited in GenBank are available in https://pubmlst.org/ campylobacter/.

Competing interests

The authors declare that they have no competing interests or personal relationships that could have influenced the work reported in this paper.

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Figures

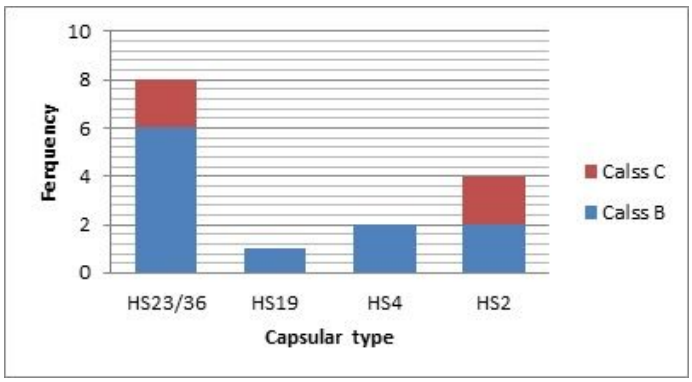
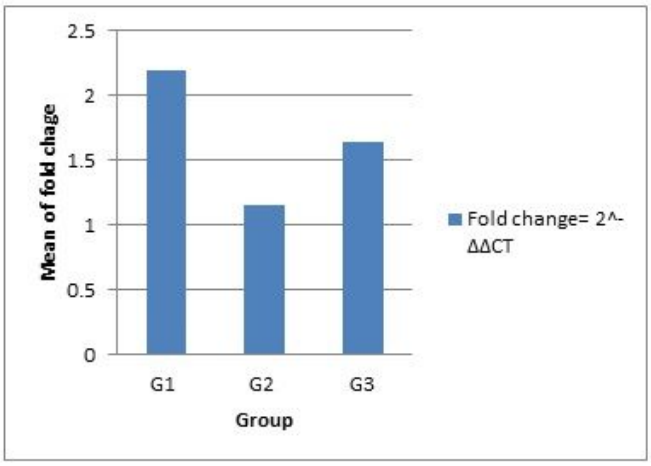


Figure 1

Relationship between presence of CPS type genes and LOS locus class genes.



G1: Isolates with selected capsular genotype and LOS class
G2: Isolates with selected capsular genotype but without sialylated LOS class
G3: Isolates without selected capsular genotype but with sialylated LOS class

Figure 2

Level of dnaK gene expression in clinical C. jejuni isolates of different groups.

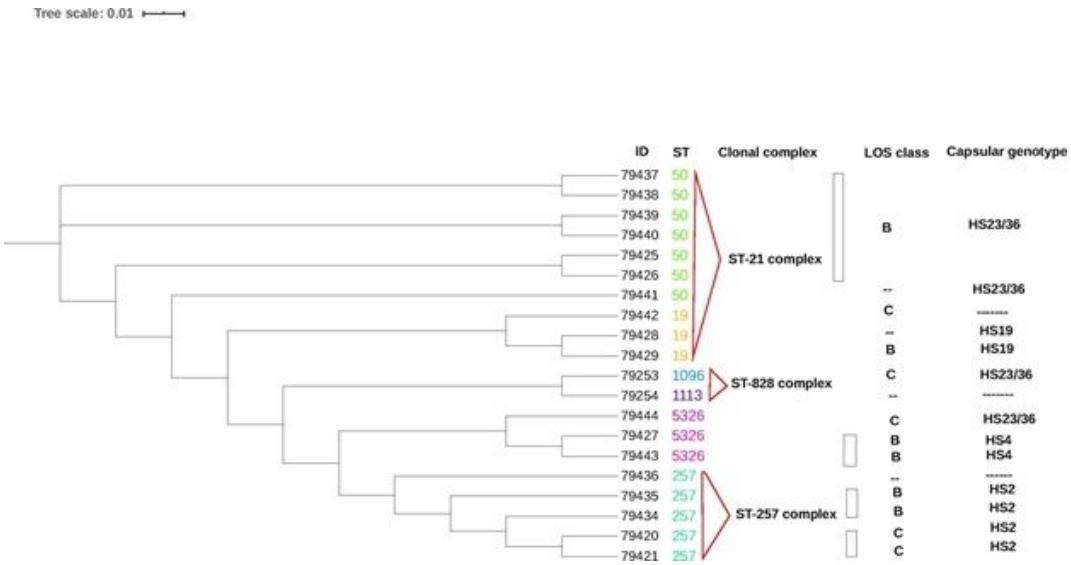


Figure 3

Dendrogram demonstrating the phylogenetic relationship between the 20 C. jejuni isolates from patients with gastroenteritis in Iran 2018 (*Dendrogran plotted by Interactive Tree of Life (iTOL) v4 [18]).

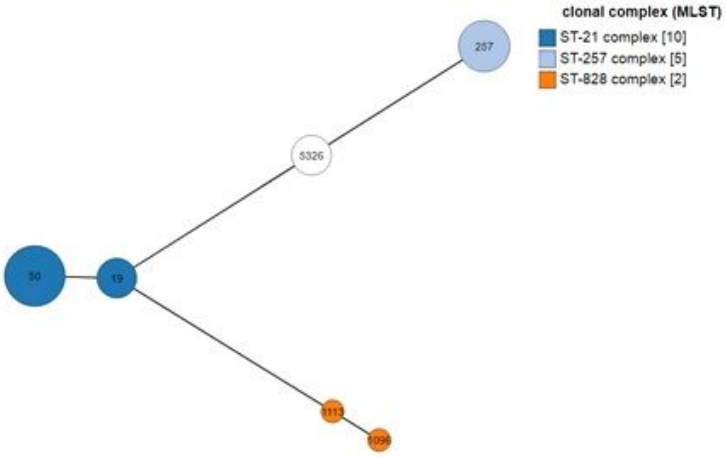
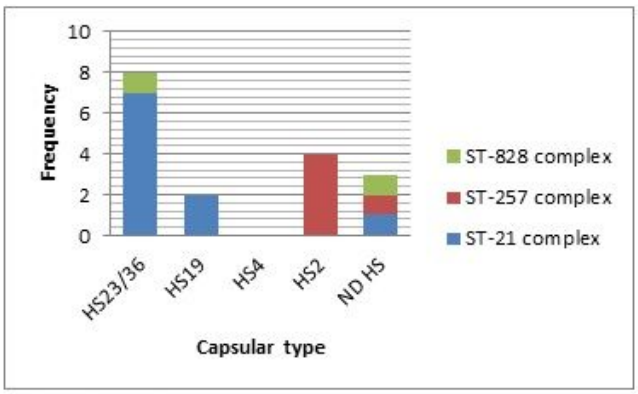


Figure 4

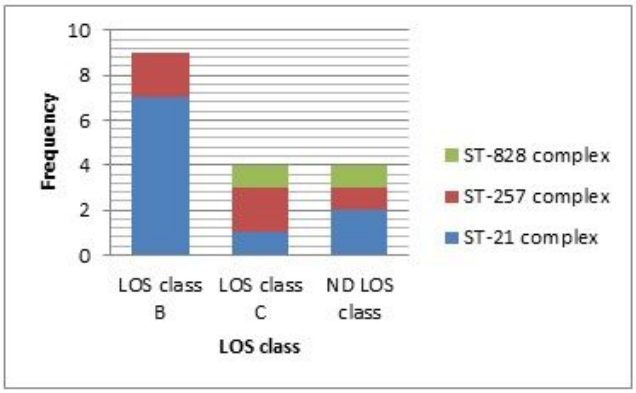
Minimum spanning tree for categorical data (based on clonal complexes) The tree was created using GrapeTree [19]. Each clonal complex is represented by a circle, numbers in each circle related to STs. the number of isolates is shown in brackets.



ND: Not determined

Figure 5

Linkage between CPS genotypes and MLST CC.



ND : Not determined

Figure 6

Linkage between LOS class and MLST CC.

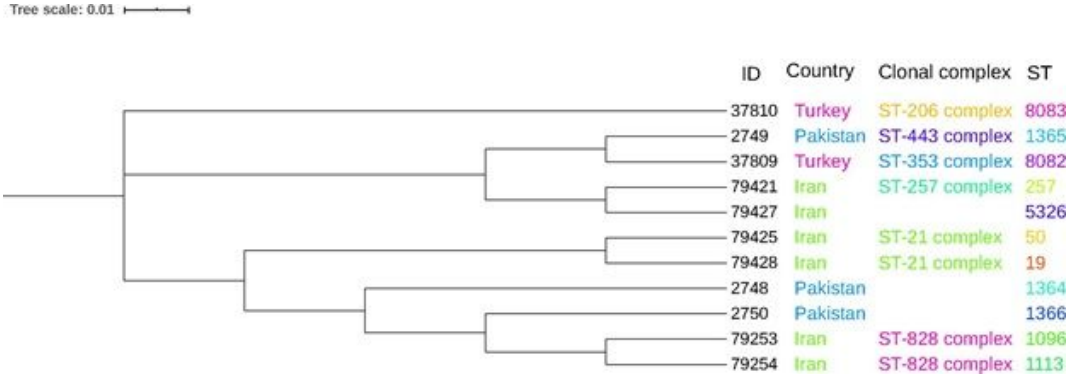


Figure 7

Phylogenetic analysis of 10 C. jejuni strains for Iran, Turkey and Pakistan. *Dendrogran plotted by Interactive Tree of Life (iTOL) v4 [18].

Figure 8

Geographic distribution of ST-19 for 1,324 Campylobacter isolates (C. jejuni (99.8%) and Campylobacter sp (0.2%) in the world, 1982-2018. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 9

Geographic distribution of ST-50 for 3,868 Campylobacter isolates (C. jejuni (99.6%) and Campylobacter sp (0.4%) in the world, 1980-2018. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 10

Geographic distribution of ST-257 for 2,689Campylobacter isolates (C. jejuni (100.0%) in the world, 1990-2018. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

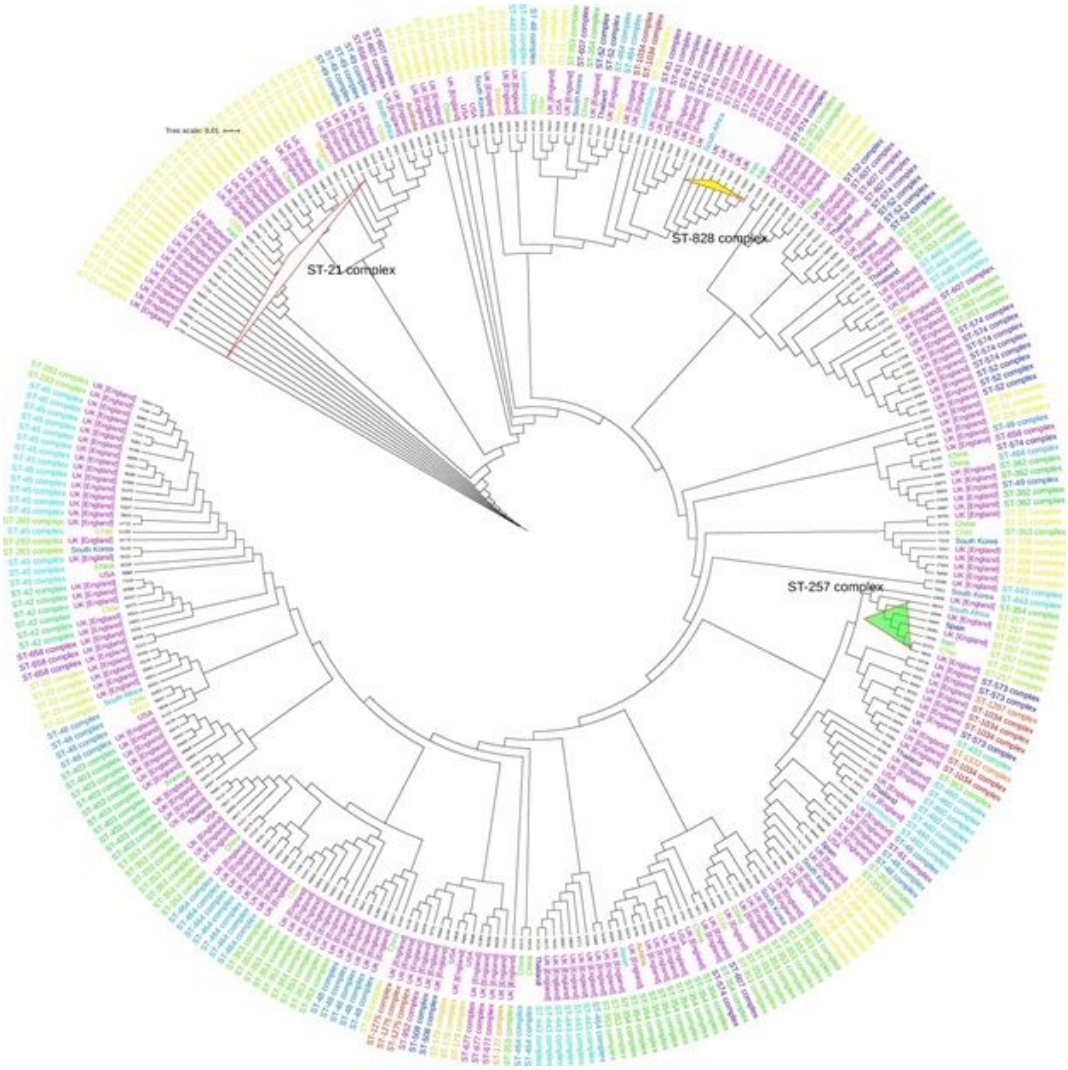


Figure 11

Phylogenetic analysis of 304 C. jejuni isolates worldwide from PubMLST database. *Dendrogran plotted by Interactive Tree Of Life (iTOL) v4 [18]. White triangle: Countries with similar CC21, yellow triangle: Countries with CC828 and green triangle: Countries with similar CC257 with Iran.