Eur J Clin Microbiol Infect Dis (2014) 33:2173–2181
DOI 10.1007/s10096-014-2184-x

ARTICLE

Comparative population structure analysis of *Campylobacter jejuni* from human and poultry origin in Bangladesh

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Received: 2 June 2014 / Accepted: 9 June 2014 / Published online: 26 June 2014

Abstract *Campylobacter jejuni* is the most important cause of antecedent infections leading to Guillain–Barré syndrome (GBS) and Miller Fisher syndrome (MFS). The objective of the present study was to define the genetic diversity, population structure, and potential role of poultry in the transmission of *Campylobacter* to humans in Bangladesh. We determined the population structure of *C. jejuni* isolated from poultry (n=66) and patients with enteritis (n=39) or GBS (n=10). Lipooligosaccharide (LOS) typing showed that 50/66 (76 %) *C. jejuni* strains isolated from poultry could be assigned to one of five LOS locus classes (A–E). The distribution of neuropathy-associated LOS locus classes A, B, and C were 30/50 (60 %) among the typable strains isolated from poultry. The LOS locus classes A, B, and C were significantly associated with GBS and enteritis-related *C. jejuni* strains more than for the poultry strains [(31/38 (82 %) vs. 30/50 (60 %), p<0.05]. Multilocus sequence typing (MLST) defined 15 sequence types (STs) and six clonal complexes (CCs) among poultry isolates, including one ST-3740 not previously documented. The most commonly identified type, ST-5 (13/66), in chicken was seen only once among human isolates (1/49) (p<0.001). Amplified fragment length polymorphism (AFLP) revealed three major clusters (A, B, and C) among *C. jejuni* isolated from humans and poultry. There seems to be a lack of overlap between the major human and chicken clones, which suggests that there may be additional sources for campylobacteriosis other than poultry in Bangladesh.

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Introduction

Campylobacter jejuni infection in humans is associated with the sudden onset of fever, abdominal cramps, and bloody diarrhea [1]. In addition, *C. jejuni* has been identified as an important trigger for Guillain–Barré syndrome (GBS), the most common acute flaccid paralysis [2]. *Campylobacter* spp. constitute part of the natural intestinal flora of many mammalian species and birds [3]. This includes not only domestic farm animals but also pet animals, such as cats and dogs [4]. Poultry is considered to be an important source of *C. jejuni*, and transmission is thought to occur as a result of cross-contamination due to lack of hygiene or via the consumption of undercooked food [5].

Molecular typing is an important tool to describe the diversity of *Campylobacter* strains and to help understand its transmission via the food chain. Several international molecular

epidemiological studies indicate that genotypes of *C. jejuni* isolated from humans are shared with strains from poultry, indicating that poultry is an important source of *Campylobacter* [6, 7]. Various molecular typing methods are currently available to study the population structure of *Campylobacter* [8]. Multilocus sequence typing (MLST) has emerged as the state-of-the-art method for the resolution of bacterial population genetics [9, 10] and is now recognized as one of the gold standard typing methods for phylogenetic studies [11]. This technique is highly reproducible, and the results are easy to interpret and can be shared through a publicly accessible database [12, 13]. Conversely, amplified fragment length polymorphism (AFLP) also documents the contribution of accessory genetic elements next to the core genome polymorphisms as investigated by MLST [4, 14]. AFLP fingerprinting has been shown to have high potential for strain identification [4, 15–18].

The pathway from *C. jejuni* infection to the development of GBS is poorly understood, but it has been repeatedly stated that interplay between bacterial and host risk factors are important triggering factors. The development of GBS after *C. jejuni* infection is related to sialylated lipooligosaccharides (LOS) on the cell surface of *C. jejuni*. Comparison of the genetic LOS loci of various *C. jejuni* strains demonstrated that three LOS locus classes (A, B, and C) were associated with the development of GBS and MFS [19]. Recently, we reported a high frequency of the axonal variant of GBS in Bangladesh, associated with preceding *C. jejuni* infection (57 %) [20]. Our earlier studies revealed that exclusive genotypes associated with either enteritis or GBS strains could not be identified. However, sialylation of LOS was significantly associated with strains isolated from GBS patients [21]. No information is available regarding the potential role of poultry in the transmission of *Campylobacter* to humans in Bangladesh. We studied here the genetic diversity among *C. jejuni* from humans and poultry with regard to LOS locus class, sequence types (STs) and clonal complexes (CCs), and AFLP types.

Materials and methods

Bacterial isolates

Sixty-six *C. jejuni* isolated between January and June 2007 from chicken cloacae in the Dhaka area of Bangladesh were included in the present study. The chickens originated from diverse geographic locales in the Dhaka region and were randomly selected. All isolates were cultured and identified to the genus and species levels, as previously described [22]. In addition, ten *C. jejuni* strains isolated from stool specimens of GBS patients and 39 *C. jejuni* from enteritis patients were included [21]. All GBS patients fulfilled the diagnostic criteria of the National Institute of Neurological Disorders and Stroke (NINDS) [23]. Bacteria were grown on blood agar plates with 5 % sheep blood at 37 °C for 48 h under microaerophilic conditions, with 6 % O_2, 7 % CO_2, 80 % N_2, and 7 % H_2 using the Anoxomat system (Anoxomat™ Mark II, Drachten, the Netherlands). Bacteria were stored at −80 °C in 15 % glycerol in brain heart infusion broth.

Bacterial DNA isolation

Genomic DNA was isolated with the Qiagen Genomic DNA purification kit according to the manufacturer's instructions (Qiagen, Venlo, the Netherlands).

Determination of the LOS locus class

To determine the LOS locus class in *C. jejuni* strains, we used specific primer sets for the classes A/B, C, D, and E, based on the DNA sequence of genes unique to the respective LOS locus class(es) described earlier [24]. To differentiate between classes A and B, we used a primer set that was based on the DNA sequence of *orf5-II* [24]. Polymerase chain reaction (PCR) assays were performed using standard conditions [24].

MLST

MLST was performed as described earlier by Dingle et al. [10]. Where no amplification product was observed on agarose gel electrophoresis, the reaction was repeated while substituting the primers by those described by Miller et al. [12]. The same primers were used for nucleotide sequencing and sequences were deposited in the pubMLST *Campylobacter* database (http://pubmlst.org/campylobacter/), enabling the identification and assignment of STs and CC. Related STs were assigned to CCs as described by Dingle et al. [10, 11]. Novel alleles and allelic profiles were submitted to http://pubmlst.org/campylobacter/ and assigned new numbers.

AFLP analysis

All *C. jejuni* strains were typed by AFLP according to previously published protocols [25]. Fingerprints were collected by fluorography and interpreted with the ABI GeneScan software (PE Applied Biosystems). Gels were normalized using an internal ROX-labeled size standard included in each lane. Densitometric curves were processed with the GelCompar version 4.1 software (Applied Maths, Kortrijk, Belgium). After normalization and background subtraction, the levels of genetic similarity between AFLP patterns were calculated with the Pearson product–moment correlation coefficient (r).

Data analysis

Sequences for each of seven alleles for all STs were concatenated and used to construct genealogies using two methods for inferring evolutionary relationships among *C. jejuni* STs. First, the relatedness of isolates was represented by a dendrogram constructed by cluster analysis using the unweighted pair group method with arithmetic means (UPGMA) in the program Start2, available at http://pubmlst.org/software/analysis/start2/ [26]. The second phylogenetic analysis estimated the clonal genealogy of STs using the model-based approach to determine bacterial microevolution (ClonalFrame 1.0). This is a model that calculates clonal relationships with improved accuracy, as it distinguishes point mutations from chromosomal recombination events. The consensus trees represent combined data from three independent runs with 50 % majority rule consensus required for the inference of relatedness. Statistical analysis was performed with Epi Info (version 3.0) using 2×2 contingency tables. Fisher's exact tests were executed and two-sided *p*-values determined. Associations were considered significant at $p<0.05$.

Results

LOS locus class diversity

In this study, 50/66 (76 %) *C. jejuni* strains isolated from poultry could be assigned to one of the five known LOS locus classes by PCR (A–E). Among the typeable strains isolated from poultry, LOS locus classes A, B, and C were 1, 27, and 2, respectively (Fig. 1). Potentially neuropathy-associated LOS locus classes A, B, and C were predominant (30/50, 60 %) (Fig. 1). The prevalence of LOS locus class E was 20/50 (40 %) and no LOS locus class D was found. Strains carrying the LOS locus classes A, B, and C were more frequent in humans than in poultry [31/38 (82 %) vs. 30/50 (60 %); $p<0.05$]. However, LOS class E was seen more often in poultry strains than in human isolates [20/50 (40 %) vs. 7/38 (18 %); $p<0.05$].

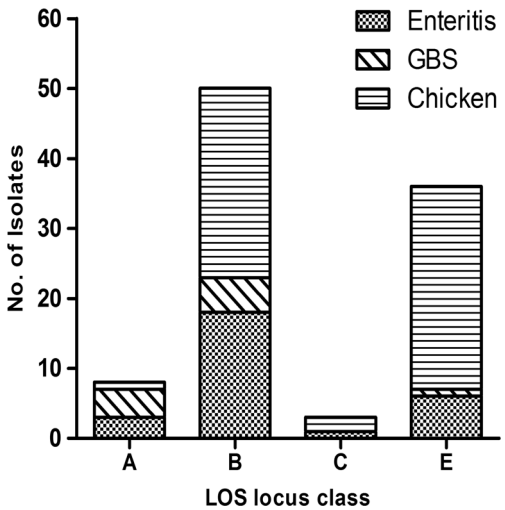


Fig. 1 Distribution of lipooligosaccharide (LOS) locus class (A, B, C, E) among the Guillain–Barré syndrome (GBS) and enteritis patients, and poultry

Diversity of MLST STs and CCs in poultry

Fifteen MLST STs were identified among *C. jejuni* isolated from poultry in Bangladesh. Three of the STs (ST-824, ST-2895, and ST-3219), were represented by single isolates, while 12 STs included between 2 and 13 isolates (Table 1). ST-5 was the most frequent among poultry isolates ($n=13$), followed by ST-1042 ($n=9$), ST-3740 ($n=8$), ST-849 ($n=6$), ST-3741 ($n=6$), ST-305 ($n=4$), ST-3744 ($n=4$), ST-3739 ($n=4$), ST-1374 ($n=3$), ST-3746 ($n=2$), ST-3745 ($n=2$), and ST-3738 ($n=2$) (Table 1). Overall, ST-3740 representing eight isolates were not reported previously. The 11 STs were grouped into six previously defined CCs. However, four STs featured in 21 (32 %) isolates could not yet be grouped into defined CCs. CC-353 encompassed the largest number of isolates ($n=19$, 29 %), followed by CC-354 ($n=15$, 23 %), CC-574 ($n=5$, 8 %), and CC-460 ($n=4$, 6 %).

The distribution of the *C. jejuni* isolated from enteritis, GBS, and poultry was investigated by a clonal frame tree demonstrating the genetic relationship of STs (Fig. 2). The distribution of STs from human and poultry revealed that 41 % of all isolates from humans overlapped with poultry isolates. The most frequent ST-5 (13/66) was predominantly associated with poultry isolates when compared to human isolates (1/49) ($p<0.001$) (Fig. 3). However, ST-3219 was significantly associated with GBS-/enteritis-related *C. jejuni* strains (13/49) when compared to poultry isolates (1/66) ($p<0.001$) (Fig. 3).

AFLP

AFLP fingerprints were identified as distinct types when band patterns shared less than 90 % similarity [14]. At a cut-off of 90 %, clustering yielded 15 different AFLP types among the 66 *C. jejuni* isolated from poultry (Fig. 4). The largest cluster (AF-1) among chicken strains consisted of 16 (24 %) isolates; other predominant clusters were AF-2 (14, 21 %) and AF-3 (9, 14 %). In addition, there were nine small clusters that each contained two to five isolates isolated from poultry. Three AFLP fingerprints of poultry isolates (ZCC-24, ZCC-324, and ZCC-382) were unique (Fig. 4). In a further effort to determine whether the AFLP clusters could be separated into source-specific groups, we compared all poultry isolates with GBS and enteritis isolates (Fig. 4). The analysis showed three major clusters (A, B, and C); all the poultry isolates ($n=66$) belonged to one cluster (B); the other two (A and C) contained human isolates only. Fisher's exact tests demonstrated a

Table 1 Multilocus sequence typing (MLST) analysis of the *Campylobacter jejuni* strains from Guillain–Barré syndrome (GBS) and enteritis patients, and poultry in Bangladesh

ST[a]	CC[b,c]	Frequency			MLST allelic profile[d]						
		GBS (n=10)	Enteritis (n=39)	Poultry (n=66)	*aspA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	*uncA*
5	353	0	1	13	7	2	5	2	10	3	6
22	22	1	0	0	1	3	6	4	3	3	3
27	362	0	2	0	1	2	42	85	11	9	8
305	574	0	0	4	9	53	2	10	11	3	3
354	354	0	1	0	8	10	2	2	11	12	6
587	362	2	1	0	1	2	42	4	90	25	8
588	NA	0	1	0	1	82	5	90	2	88	1
660	22	1	0	0	1	3	6	4	54	91	3
824	257	0	0	1	9	2	2	2	11	5	6
849	NA	0	0	6	55	21	27	10	147	37	6
985	403	1	1	0	10	27	89	19	10	132	7
1042	354	0	0	9	8	10	2	2	67	12	6
1374	NA	0	3	3	24	2	5	72	2	5	6
1323	353	0	1	0	7	17	5	10	11	3	6
1377	42	0	1	0	1	2	42	4	153	9	8
2109	45	1	1	0	4	7	10	4	10	7	1
2895	574	0	0	1	9	53	5	10	11	3	3
2993	362	0	2	0	1	2	42	4	11	9	8
3219	403	4	9	1	10	27	33	19	10	5	7
3632	NA	0	3	0	91	2	42	4	169	25	8
3738	460	0	0	2	24	30	2	2	2	120	6
3739	NA	0	0	4	7	4	5	68	11	1	6
3740	NA	0	0	8	7	4	5	68	11	1	**257**
3741	354	0	3	6	234	10	2	2	67	12	6
3742	NA	0	2	0	1	308	95	49	436	353	258
3743	NA	0	1	0	233	2	42	4	90	25	8
3744	353	0	0	4	7	2	5	341	10	3	6
3745	460	0	0	2	24	30	255	2	89	59	6
3746	353	0	0	2	7	84	5	10	437	3	6
3748	41	0	2	0	235	2	42	62	11	9	8
3968	NA	0	1	0	8	2	52	68	11	5	7
3969	353	0	1	0	7	2	33	2	10	3	6
3970	45	0	1	0	4	7	10	249	3	7	1

[a] *ST* sequence type; the MLST ST first reported in this study is indicated in bold

[b] *CC* clonal complex

[c] *NA* not assigned

[d] The new allele identified in this study is in bold

significant association between AFLP cluster and the sources (p<0.001) (data not shown).

Correlation between sequence typing and LOS classes with AFLP typing

Figure 4 represents the relation between the *C. jejuni* LOS locus classes determined by PCR and certain MLST STs. No correlation was found between STs and the LOS locus classes of the *C. jejuni* strains from human and poultry sources. LOS locus class B featured across 16 different STs, and LOS class E represented 11 different STs in *C. jejuni* from human and poultry sources. The correlation between sequence typing and LOS class by AFLP typing was also found to be heterogeneous (Fig. 4). *C. jejuni* with LOS class B was distributed across all three clusters (A, B, and C), whereas LOS class E

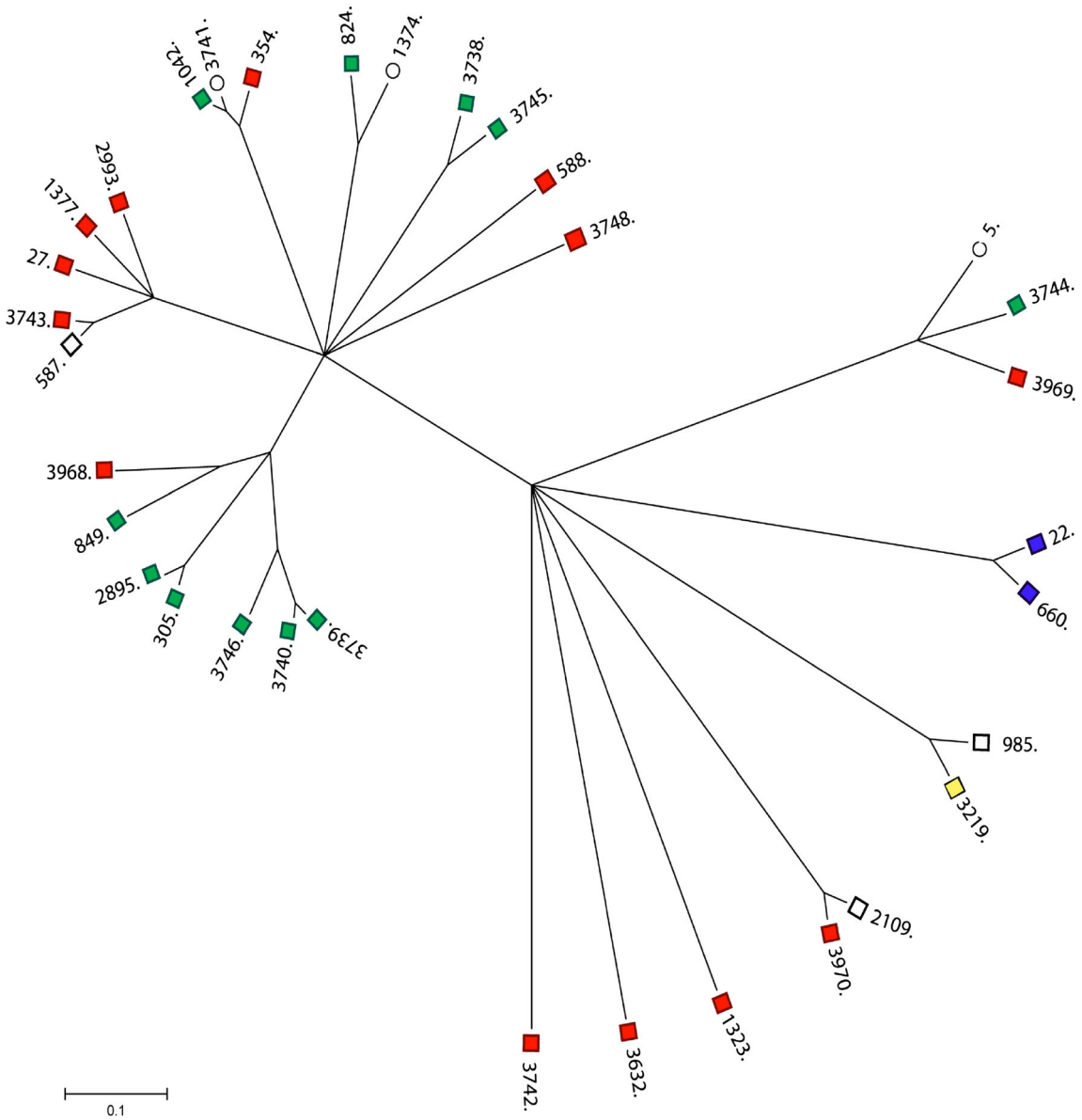


Fig. 2 Clonal frame tree demonstrating the genetic relationships of sequence types (STs) between *Campylobacter jejuni* isolated from poultry and patients with gastroenteritis (GI) and Guillain–Barré syndrome (GBS) in Bangladesh. Chicken isolates are shown as *green boxes*, GI strains as *red boxes*, GBS isolates are highlighted by the use of *blue boxes*, those strains found to be associated with both poultry and GI are indicated by *white circles*, those associated with GI and GBS can be identified by *white boxes*, and, finally, those isolates associated with poultry, GI, and GBS are shown by *orange boxes*

was grouped mostly (n=24, 89 %) in one cluster (B). We found 14 different STs in cluster A and two STs in cluster C. ST-3219 (12/14) was predominant in cluster C. Cluster B contained the other predominant STs, such as ST-5 (n=14), ST-3741 (n=9), ST-1042 (n=9), ST-3740 (n=8), and ST-3744 (n=4).

Discussion

In an earlier study, we reported an unusually high frequency of the axonal variant of GBS associated with preceding *C. jejuni* infection in Bangladesh [20]. Although a significant association between GBS and preceding *C. jejuni* infection was found, we could not determine the sources of infection and the route of transmission of *C. jejuni*. Poultry is usually identified as the most common reservoir of *Campylobacter*. We performed comparative genotyping of *C. jejuni* isolated from poultry and patients with enteritis and GBS by LOS class PCR typing, MLST, and AFLP fingerprinting. Our results differ extensively from those presented in an earlier study from developed countries [27], where it was reported that most *C. jejuni* isolates from poultry products were identified as LOS locus class C. We found that the largest numbers of

isolates were in other LOS classes, and less than 2 % expressed LOS locus class C. Nevertheless, a high prevalence of LOS locus classes A, B, and C (60 %) was found in strains from poultry. Several molecular subtyping studies revealed that the population structure of *C. jejuni* seems highly diverse and weakly clonal [10, 28], which we confirmed for *C. jejuni* strains isolated from humans and poultry in Bangladesh. There seems to be a similar partial overlap between genotypes isolated from poultry and enteritis patients. Interestingly, no overlap was documented between *C. jejuni* genotypes encountered in GBS patients and in poultry. However, the number of GBS-related *C. jejuni* is too small to allow for definite conclusions to be drawn.

The analysis of LOS locus classes supports the observation that the distribution of *C. jejuni* found in poultry differs from the distribution in *C. jejuni* isolates from patients with enteritis and GBS [24]. Consistent with our results, Dingle et al. [10] identified six CCs in 34 isolates obtained from poultry. Kinana et al. [29] found seven CCs in 46 isolates from poultry in Senegal. In contrast to previous studies in which the ST-21 complex was the largest complex [10, 11, 30], this complex appears to be uncommon among *Campylobacter* poultry isolates from Bangladesh. However, the ST-21 complex is widespread and has been reported in a variety of hosts and has previously been described to be associated with infections in humans, livestock, and also with environmental sources [10, 31]. ST-5 (ST-353 complex), the most common ST in poultry isolates from Bangladesh, was also shown to be common in Senegalese poultry [29]. We recently reported that ST-3219 (ST-403 complex) is prevalent among GBS and enteritis-related *C. jejuni* from Bangladesh [28]. Our comparative genotyping analysis suggests that ST-3219 isolates are not common in poultry in Bangladesh. ST-3219 has been demonstrated in *C. jejuni* isolated from dogs in England [32], porcine, and cattle isolates [33–35]. This ST has also been observed in humans previously and was reported to be the dominant genotype in a study in Curacao [10, 11, 14]. In a large study carried out in New Zealand, McTavish et al. concluded that ST-3219 strains were predominantly linked to cattle but not to poultry [36].

Fig. 4 Combined dendrogram based on amplified fragment length polymorphism (AFLP) band patterns of *Campylobacter jejuni* human and poultry isolates. The percentage of genetic homology between banding patterns is indicated. Sources, sequence types (STs), lipooligosaccharide (LOS) types, and AFLP types are plotted next to the dendrogram

We also used AFLP analysis, as this method appears to be an excellent tool for assessing the population structure of *C. jejuni*. This analysis revealed that a distinct subpopulation of *C. jejuni* is associated with either poultry or humans. Though source-specific clusters were identified, the AFLP types of all *C. jejuni* appeared to be heterogeneous, and no specific AFLP type infecting chickens or humans specifically was identified. The high diversity of AFLP fingerprints is a reflection of the normal genetic diversity among *C. jejuni* [25]. Earlier studies also revealed that MLST, AFLP, PFGE, and DNA microarray were unsuccessful to identify GBS-specific genetic markers by comparing the genomes of *C. jejuni* [11, 25, 37, 38].

This study has several limitations: first, the number of isolates from poultry was relatively small and the resulting limiting power may leave true associations undetected; second, although we sampled poultry from a different geographical area, their precise origin was unknown; larger follow-up studies should include GPS mapping of all poultry and sample locations; third, as we included only one bacterial colony per chicken sample, we cannot exclude the presence of more than one *Campylobacter* genotype/phenotype in individual chickens.

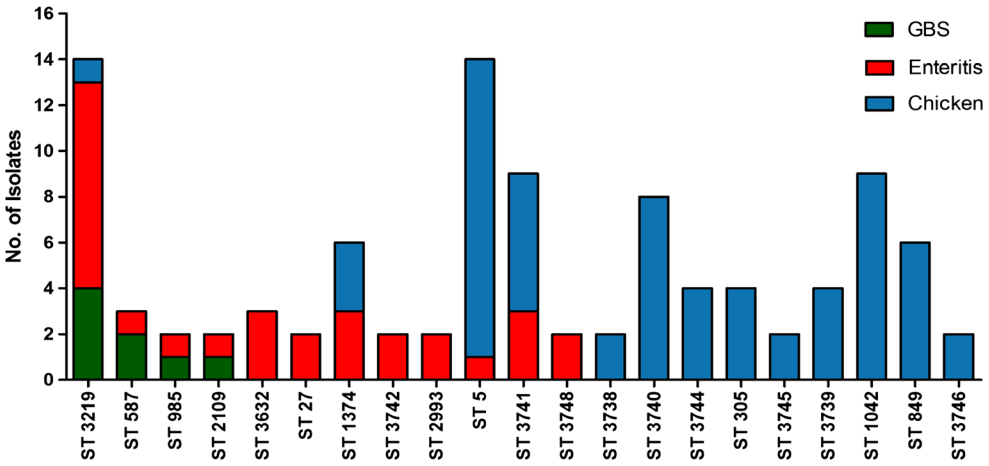
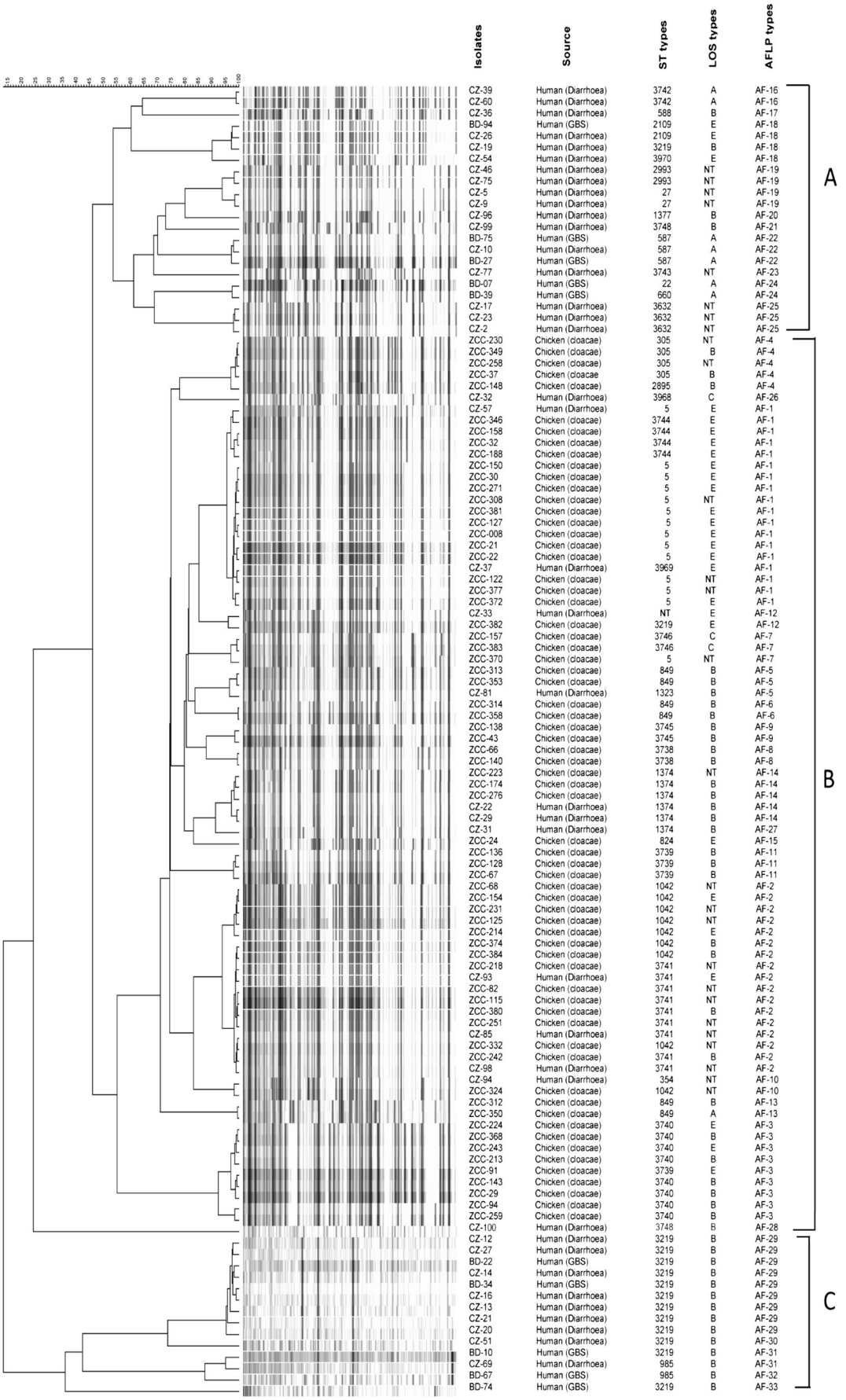


Fig. 3 Distribution of sequence types (STs) among *Campylobacter jejuni* isolated from Guillain–Barré syndrome (GBS) and enteritis patients and poultry in Bangladesh. Twelve STs contained one isolate each and did not overlap with any sources, and are not included in the figure (chicken: ST-2895, ST-824; GBS: ST-22, ST-660; enteritis: ST-354, ST-588, ST-1323, ST-1377, ST-3968, ST-3969, ST-3970, ST-3743)



Isolates	Source	ST types	LOS types	AFLP types	
CZ-39	Human (Diarrhoea)	3742	A	AF-16	A
CZ-60	Human (Diarrhoea)	3742	A	AF-16	
CZ-36	Human (Diarrhoea)	588	B	AF-17	
BD-94	Human (GBS)	2109	E	AF-18	
CZ-26	Human (Diarrhoea)	2109	E	AF-18	
CZ-19	Human (Diarrhoea)	3219	B	AF-18	
CZ-54	Human (Diarrhoea)	3970	E	AF-18	
CZ-46	Human (Diarrhoea)	2993	NT	AF-19	
CZ-75	Human (Diarrhoea)	2993	NT	AF-19	
CZ-5	Human (Diarrhoea)	27	NT	AF-19	
CZ-9	Human (Diarrhoea)	27	NT	AF-19	
CZ-96	Human (Diarrhoea)	1377	B	AF-20	
CZ-99	Human (Diarrhoea)	3748	B	AF-21	
BD-75	Human (GBS)	587	A	AF-22	
CZ-10	Human (Diarrhoea)	587	A	AF-22	
BD-27	Human (GBS)	587	A	AF-22	
CZ-77	Human (Diarrhoea)	3743	NT	AF-23	
BD-07	Human (GBS)	22	A	AF-24	
BD-39	Human (GBS)	660	A	AF-24	
CZ-17	Human (Diarrhoea)	3632	NT	AF-25	
CZ-23	Human (Diarrhoea)	3632	NT	AF-25	
CZ-2	Human (Diarrhoea)	3632	NT	AF-25	
ZCC-230	Chicken (cloacae)	305	NT	AF-4	B
ZCC-349	Chicken (cloacae)	305	B	AF-4	
ZCC-258	Chicken (cloacae)	305	NT	AF-4	
ZCC-37	Chicken (cloacae	305	B	AF-4	
ZCC-148	Chicken (cloacae)	2895	B	AF-4	
CZ-32	Human (Diarrhoea)	3968	C	AF-26	
CZ-57	Human (Diarrhoea)	5	E	AF-1	
ZCC-346	Chicken (cloacae)	3744	E	AF-1	
ZCC-158	Chicken (cloacae)	3744	E	AF-1	
ZCC-32	Chicken (cloacae)	3744	E	AF-1	
ZCC-188	Chicken (cloacae)	3744	E	AF-1	
ZCC-150	Chicken (cloacae)	5	E	AF-1	
ZCC-30	Chicken (cloacae)	5	E	AF-1	
ZCC-271	Chicken (cloacae)	5	E	AF-1	
ZCC-308	Chicken (cloacae)	5	NT	AF-1	
ZCC-381	Chicken (cloacae)	5	E	AF-1	
ZCC-127	Chicken (cloacae)	5	E	AF-1	
ZCC-008	Chicken (cloacae)	5	E	AF-1	
ZCC-21	Chicken (cloacae)	5	E	AF-1	
ZCC-22	Chicken (cloacae)	5	E	AF-1	
CZ-37	Human (Diarrhoea)	3969	E	AF-1	
ZCC-122	Chicken (cloacae)	5	NT	AF-1	
ZCC-377	Chicken (cloacae)	5	NT	AF-1	
ZCC-372	Chicken (cloacae)	5	E	AF-1	
CZ-33	Human (Diarrhoea)	NT	E	AF-12	
ZCC-382	Chicken (cloacae)	3219	E	AF-12	
ZCC-157	Chicken (cloacae)	3746	C	AF-7	
ZCC-383	Chicken (cloacae)	3746	C	AF-7	
ZCC-370	Chicken (cloacae)	5	NT	AF-7	
ZCC-313	Chicken (cloacae)	849	B	AF-5	
ZCC-353	Chicken (cloacae)	849	B	AF-5	
CZ-81	Human (Diarrhoea)	1323	B	AF-5	
ZCC-314	Chicken (cloacae)	849	B	AF-6	
ZCC-358	Chicken (cloacae)	849	B	AF-6	
ZCC-138	Chicken (cloacae)	3745	B	AF-9	
ZCC-43	Chicken (cloacae)	3745	B	AF-9	
ZCC-66	Chicken (cloacae)	3738	B	AF-8	
ZCC-140	Chicken (cloacae)	3738	B	AF-8	
ZCC-223	Chicken (cloacae)	1374	NT	AF-14	
ZCC-174	Chicken (cloacae)	1374	B	AF-14	
ZCC-276	Chicken (cloacae)	1374	B	AF-14	
CZ-22	Human (Diarrhoea)	1374	B	AF-14	
CZ-29	Human (Diarrhoea)	1374	B	AF-14	
CZ-31	Human (Diarrhoea)	1374	B	AF-27	
ZCC-24	Chicken (cloacae)	824	E	AF-15	
ZCC-136	Chicken (cloacae)	3739	B	AF-11	
ZCC-128	Chicken (cloacae)	3739	B	AF-11	
ZCC-67	Chicken (cloacae)	3739	B	AF-11	
ZCC-68	Chicken (cloacae)	1042	NT	AF-2	
ZCC-154	Chicken (cloacae)	1042	E	AF-2	
ZCC-231	Chicken (cloacae)	1042	NT	AF-2	
ZCC-125	Chicken (cloacae)	1042	NT	AF-2	
ZCC-214	Chicken (cloacae)	1042	E	AF-2	
ZCC-374	Chicken (cloacae)	1042	B	AF-2	
ZCC-384	Chicken (cloacae)	1042	B	AF-2	
ZCC-218	Chicken (cloacae)	3741	NT	AF-2	
CZ-93	Human (Diarrhoea)	3741	E	AF-2	
ZCC-82	Chicken (cloacae)	3741	NT	AF-2	
ZCC-115	Chicken (cloacae)	3741	NT	AF-2	
ZCC-380	Chicken (cloacae)	3741	B	AF-2	
ZCC-251	Chicken (cloacae)	3741	NT	AF-2	
CZ-85	Human (Diarrhoea)	3741	NT	AF-2	
ZCC-332	Chicken (cloacae)	1042	NT	AF-2	
ZCC-242	Chicken (cloacae)	3741	B	AF-2	
CZ-98	Human (Diarrhoea)	3741	NT	AF-2	
CZ-94	Human (Diarrhoea)	354	NT	AF-10	
ZCC-324	Chicken (cloacae)	1042	NT	AF-10	
ZCC-312	Chicken (cloacae)	849	B	AF-13	
ZCC-350	Chicken (cloacae)	849	A	AF-13	
ZCC-224	Chicken (cloacae)	3740	E	AF-3	
ZCC-368	Chicken (cloacae)	3740	B	AF-3	
ZCC-243	Chicken (cloacae)	3740	E	AF-3	
ZCC-213	Chicken (cloacae)	3740	B	AF-3	
ZCC-91	Chicken (cloacae)	3739	E	AF-3	
ZCC-143	Chicken (cloacae)	3740	B	AF-3	
ZCC-29	Chicken (cloacae)	3740	B	AF-3	
ZCC-94	Chicken (cloacae)	3740	B	AF-3	
ZCC-259	Chicken (cloacae)	3740	B	AF-3	
CZ-100	Human (Diarrhoea)	3748	B	AF-28	
CZ-12	Human (Diarrhoea)	3219	B	AF-29	C
CZ-27	Human (Diarrhoea)	3219	B	AF-29	
BD-22	Human (GBS)	3219	B	AF-29	
CZ-14	Human (Diarrhoea)	3219	B	AF-29	
BD-34	Human (GBS)	3219	B	AF-29	
CZ-16	Human (Diarrhoea)	3219	B	AF-29	
CZ-13	Human (Diarrhoea)	3219	B	AF-29	
CZ-21	Human (Diarrhoea)	3219	B	AF-29	
CZ-20	Human (Diarrhoea)	3219	B	AF-29	
CZ-51	Human (Diarrhoea)	3219	B	AF-30	
BD-10	Human (GBS)	3219	B	AF-31	
CZ-69	Human (Diarrhoea)	985	B	AF-31	
BD-67	Human (GBS)	985	B	AF-32	
BD-74	Human (GBS)	3219	B	AF-33	

In conclusion, there appears to be considerable genetic diversity among *C. jejuni* strains obtained from poultry and humans in Bangladesh. Future efforts should be aimed to address whether the presence of sources other than poultry play a role in the epidemiology of enteritis and GBS in Bangladesh.

Acknowledgments This research was funded by the Erasmus University Medical Center, Rotterdam, the Netherlands and the International Centre for Diarrheal Disease Research, Dhaka, Bangladesh (ICDDR,B). The ICDDR,B acknowledges with gratitude the commitment of the Government of Bangladesh to the Centre's research efforts. The ICDDR,B also gratefully acknowledges the following donors which provide unrestricted support to the Centre's research efforts: Australian Agency for International Development (AusAID), Government of the People's Republic of Bangladesh, Canadian International Development Agency (CIDA), Swedish International Development Cooperation Agency (Sida), and the Department for International Development (DFID), UK. We are indebted to all the neurologists who referred their patients to us.

Conflict of interest The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflict of interest.

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