CRISPR typing of *Campylobacter jejuni* reveals a link between CRISPR array preservation and Guillain-Barré syndrome

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Research Article

Keywords: Guillain-Barré syndrome, Campylobacter jejuni, CRISPR typing, CRISPR array preservation

Posted Date: May 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-453681/v1

Abstract

Guillain-Barré syndrome (GBS) is a post-infection sequela of *Campylobacter jejuni*-induced enteritis. Clustered regularly interspaced short palindromic repeats and associated genes (CRISPR-Cas) confers adaptive immunity and plays role in virulence in many bacteria. We investigated *C. jejuni* CRISPR type (CT) to explore association of CRISPR-Cas with risk of developing GBS. We analysed CRISPR-*Cas* in *C. jejuni* isolated from 30 patients with GBS, 60 patients with enteritis and 52 healthy controls from Bangladesh. CRISPR types were determined by PCR followed by CRISPR array sequencing. Statistical and genomic analyses were performed using SPSS and multiple web-based software respectively. We found preserved CRISPR array was significantly more frequent in GBS-related strains than healthy control-related strains (P = 0.02, OR = 2.95). Increased CRISPR array length was significantly associated with GBS compared to healthy control- (P = 0.003, AUC = 0.7) and enteritis-related strains (P = 0.02, AUC = 0.65). We reported 38 new CT found among 70 CRISPR-preserving strains. CT of GBS-related strains were unique from enteritis- and healthy control-related strains. Eighty spacers, including 20 novel spacers, were identified among the CRISPR-preserving strains. CRISPR typing had more discriminatory power than PCR-based subtyping in enteritis- and healthy control-related strains. Further genomic analyses are warranted to elucidate role of the *C. jejuni* CRISPR array in GBS pathogenesis.

Introduction

Campylobacter jejuni is a leading cause of bacterial gastroenteritis worldwide and the most frequent antecedent event associated with the post-infection sequela, Guillain-Barré syndrome (GBS) [1–3]. Molecular mimicry between bacterial lipopolysaccharide (LPS) and host nerve gangliosides is the apparent cause of pathogen-derived GBS in a susceptible host [4,5]. However, not all *C. jejuni*-infected individuals develop GBS after enteritis [6]. In addition to host susceptibility factors [7–10], bacterial virulence factors also function as key regulatory elements that may trigger GBS [11–13]. Moreover, the *cst-II* sialyltransferase gene of *C. jejuni*, which encodes ganglioside-like structures expressed on the bacterial cell surface, has been linked to GBS [14]. In addition to disease pathogenesis, *cst-II* has been shown to confer efficient bacteriophage resistance in *C. jejuni* along with a functionally complex adaptive immune system, the type II Clusters Regularly Interspaced Short Palindromic Repeat and associated genes (CRISPR-Cas) [15].

The CRISPR-Cas system is a repeat region of numerous archaeal and bacterial DNA sequences that provides adaptive immunity against invading phages and plasmids by targeting their nucleic acids in a sequence-specific manner [16–18]. The latest CRISPR-Cas system classification includes two major classes, six types and 33 subtypes based on organizational variation in the *Cas* genes [19]. The *C. jejuni* genome harbours a class 2, type II-C CRISPR-Cas system comprised of a *Cas9-Cas1-Cas2* operon followed by a trans-activating CRISPR-RNA (tracrRNA) region and CRISPR repeat-spacer array, which is transcribed in the opposite direction [20]. The CRISPR array in *C. jejuni* is composed of multiple 36-bp tandem consensus direct repeats (DRs) separated by ‘spacer’ sequences of similar length (30 bp) acquired from an invading foreign phage or plasmid [21,22].

In addition to conferring adaptive immunity against invading foreign DNA, the CRISPR-Cas system also plays a role in regulation of virulence genes, competition between mobile genetic elements (MGEs), DNA repair, gene regulation of group behaviour, and the acquisition of antibiotic resistance genes and pathogenicity islands [23,24]. The type II-C CRISPR-Cas system exerts regulatory roles in pathogenicity, cell surface immunity, stress response bacterial physiology and anti-microbial resistance in many bacteria, including *C. jejuni* [25,26].

Due to the presence of polymorphisms in the spacer alleles within the CRISPR array, CRISPR-based genotyping (CRISPR typing) of bacteria enables high-resolution genotyping of pathogenic bacteria, including *C. jejuni* isolates. CRISPR typing also provides an effective framework for epidemiologic source tracking, studies of genome microevolution and host-virus population dynamics [27,28]. A previous comparative genotyping study revealed diversity among genotypes and clonal complexes of various strains of *C. jejuni* isolated from chickens, individuals with enteritis and patients with GBS in Bangladesh [29]. Serotyping and comparative genomic analyses demonstrated certain serotypes and genotypes were overrepresented in *C. jejuni* isolates obtained from Bangladeshi patients with GBS [30,31]. Thus, this study aimed to determine the CRISPR types (CTs) in *C. jejuni*-related strains and explore the association between preservation of the CRISPR-Cas sequence and the risk of developing GBS in Bangladesh.

Results

Distribution of CRISPR-Cas components in C. jejuni

CRISPR array was detected in 67% of the GBS-related, 48% of the enteritis-related and 40% of the healthy control-related *C. jejuni* strains (Table 1). There were no significant differences in the frequencies of the Cas genes (*Cas1, Cas2* and *Cas9*) between the groups of *C. jejuni* strains related to GBS, enteritis and healthy controls. All GBS-associated strains and almost 90% of the enteritis and healthy control-related *C. jejuni* strains were positive for at least one *Cas* gene (Table 1).

Table 1
Distribution of CRISPR-Cas components in *C. jejuni* strains isolated from patients with GBS, enteritis and healthy controls. [a] Indicates significant difference of CRISPR array preservation among *C. jejuni* strains isolated from GBS and healthy controls. [b] Calculating OR was not applicable as all GBS related *C. jejuni* strains contained Cas genes (Cas1, Cas2, Cas9).

CRISPR-Cas components	Source of *C. jejuni* isolates			GBS vs. enteritis		GBS vs. healthy controls		Enteritis vs. healthy controls	
	GBS n = 30 (%)	Enteritis n = 60 (%)	Healthy controls n = 52 (%)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Preserved CRISPR array	20 (67%)	29 (48%)	21 (41%)	0.07	2.14 (0.85–5.32)	0.02[a]	2.95 (1.15–7.56)	0.26	1.38 (0.65–2.92)
Cas1	30 (100%)	53 (88%)	47 (89%)	NA[b]	NA[b]	NA[b]	NA[b]	0.59	0.97 (0.3–3.08)
Cas2	30 (100%)	54 (90%)	46 (87%)	NA[b]	NA[b]	NA[b]	NA[b]	0.4	1.34 (0.43–4.36)
Cas9	30 (100%)	54 (90%)	48 (91%)	NA[b]	NA[b]	NA[b]	NA[b]	0.58	0.94 (0.27–3.27)

CRISPR array preservation and GBS

A preserved CRISPR array was significantly more frequent among the GBS-related strains than the healthy control-related strains (Odds ratio, OR: 2.95, 95% confidence interval, CI: 1.15–7.56, *P* value = 0.02), but not the enteritis-related strains (Table 1). Longer CRISPR arrays were more common among GBS-related strains than both the healthy control-related strains (Nagelkerke R^2 = 0.142, *P* = 0.0026, area under ROC curve [AUC] = 0.70; Fig. 1a) and enteritis-related strains (Nagelkerke R^2 = 0.084, *P* = 0.018, AUC = 0.65; Fig. 1b).

CRISPR types in C. jejuni

PCR-based rapid screening revealed a total of seven CRISPR subtypes across all 142 *C. jejuni* isolates (Table 2). Amplicon lengths of 0 bp (negative in the F2 primer PCR assay) and 190 bp were not considered as subtypes, as they either did not harbour any CRISPR array or did not contain any spacer in their CRISPR array. Two subtypes were not detected in the GBS-related strains (330 bp and 720 bp; Table 2) and two subtypes were not detected in the enteritis-related strains (530 bp and 590 bp; Table 2); the other three subtypes were ubiquitously detected in the GBS-, enteritis- and healthy control-related strains.

Table 2

PCR-based detection of CRISPR subtypes in *C. jejuni* strains isolated from patients with GBS, enteritis and healthy controls. [a] Indicates PCR amplicon length in PCR assay with F2 and R primers (Fig. 4). [b] These *C. jejuni* strains do not contain any CRISPR array.

PCR amplicon length (*L* bp)[a]	CRISPR array length (*L*-155) bp	*C. jejuni* isolated from	Frequency *n* = 142 (%)	Spacer number	Repeat number
190	35	GBS	5 (3%)	0	1
		Enteritis	25 (17%)		
		Healthy controls	17 (12%)		
260	105	GBS	3 (2%)	1	2
		Enteritis	15 (10%)		
		Healthy controls	9 (6%)		
330	175	GBS	0 (0%)	2	3
		Enteritis	5 (3%)		
		Healthy controls	3 (2%)		
390	235	GBS	5 (3%)	3	4
		Enteritis	3 (2%)		
		Healthy controls	4 (3%)		
460	305	GBS	7 (5%)	4	5
		Enteritis	4 (3%)		
		Healthy controls	2 (1%)		
530	375	GBS	1 (1%)	5	6
		Enteritis	1 (1%)		
		Healthy controls	1 (1%)		
590	435	GBS	4 (3%)	6	7
		Enteritis	0 (0%)		
		Healthy controls	1 (1%)		

PCR amplicon length (*L* bp)[a]	CRISPR array length (*L*-155) bp	*C. jejuni* isolated from	Frequency *n* = 142 (%)	Spacer number	Repeat number
720	565	GBS	0 (0%)	8	9
		Enteritis	1 (1%)		
		Healthy controls	1 (1%)		
Negative[b]	Not applicable	GBS	5 (3%)	Not applicable	Not applicable
		Enteritis	6 (4%)		
		Healthy controls	14 (10%)		

Spacer polymorphism-based CRISPR typing identified seven CTs in the GBS-related strains, 22 CTs in the enteritis-related strains and 19 CTs in the healthy control-related strains (Table 3). Six CTs were found in both the enteritis- and healthy control-related strains. However, all seven CTs found in the GBS-related strains were absent in the enteritis- and healthy control-related strains (see Supplementary Table S1 online). Moreover, based on the presence of unique spacer arrangements that did not match with any *C. jejuni* CRISPR array sequence in the NCBI nucleotide database, 38 novel CTs were detected among the 42 CTs [32]. The most frequent CT among the GBS-related strains was CT 1. CT 9 was most frequent among enteritis-related strains, and CT 18 and CT 42 were most frequent among healthy control-related strains (see Supplementary Table S1 online). Spacer polymorphism-based CRISPR typing had a significantly higher discriminatory power than PCR-based typing in both the enteritis-related and healthy control-related *C. jejuni* strains (SID = 0.97 and 0.99, respectively; Table 3). However, due to the low (or lack of) variation in spacer arrangement within CRISPR arrays of the same length, PCR-based typing and sequence-based typing had similar discriminatory power among the GBS-related *C. jejuni* strains (SID = 0.79 and 0.83, respectively; Table 3).

Table 3
Discriminatory power of CRISPR typing and subtyping methods for *C. jejuni* strains isolated from patients with GBS, enteritis and healthy controls.

C. jejuni isolated from (*n*)	Frequency of *C. jejuni* isolates with preserved CRISPR array (%)	CRISPR genotyping/subtyping method	Number of found subtypes/CT	Simpson's index of diversity (SID)[d]	Jackknife CI (95%)
Patients with GBS (*30*)	20 (67%)	Sequencing	7	0.83	0.74–0.92
		PCR	5	0.79	0.70–0.88
Patients with enteritis (*60*)	29 (48%)	Sequencing	22	0.97	0.93-1.00
		PCR	5	0.73	0.59–0.85
Healthy controls (*52*)	21 (40%)	Sequencing	19	0.99	0.97-1.00
		PCR	7	0.78	0.63–0.93

CRISPR array spacer diversity

We identified a total of 80 unique spacer sequences across all 70 CRISPR array-containing *C. jejuni* strains (see Supplementary Table S2 online). Sixteen common spacers were detected in the enteritis- and healthy control-related strains. However, the GBS-related strains only contained three spacers (alleles 1, 5, 23) common to the enteritis-related strains and three spacers (alleles 1, 5, 21) common to the healthy control-related strains (see Supplementary Table S1 online). Moreover, we identified 20 previously unreported novel spacer sequences [33,34]. Six spacers (alleles 8, 23, 32, 40, 42 and 49; see Supplementary Table S2 online) contained at least one nucleotide polymorphism site. The UPGMA dendrogram revealed that the enteritis- and healthy control-related strains were more closely related than the GBS-related strains, indicating a distinctive pattern of spacer acquisition and arrangement within the CRISPR arrays of the GBS-related strains (Fig. 2).

Features of target proto-spacers

We found target proto-spacers within 62 of the 80 spacer sequences (see Supplementary Table S2 online). The target proto-spacers detected in 19 spacers from GBS-related strains, 31 spacers from enteritis-related strains and 28 spacers from healthy control-related strains were found in *Campylobacter* phages or plasmids. The most abundant proto-spacers were found in *Campylobacter phage DA10* (NCBI accession

pacers from GBS-related strains, 27 spacers from enteritis-

related strains and 26 spacers from healthy control-related strains (see Supplementary Table S1 and Supplementary Table S2 online). Nine spacers from GBS-related strains, 10 spacers from enteritis-related strains and eight spacers from healthy control-related strains shared at least 80% homology with target proto-spacers found in non-*Campylobacter* phage or plasmids (see Supplementary Table S2 online). The sequence logo (Fig. 3) created by aligning the 3` downstream proto-spacer sequences [33] identified 5 -$\mathbb{NN}AYAC$- 3 as a putative proto-spacer adjacent motif (PAM) for the *C. jejuni Cas9* endonuclease.

Discussion

This study indicates that preservation of the CRISPR array in *C. jejuni* is strongly associated with the risk of developing GBS. In addition, the probability of developing GBS following *C. jejuni* infection increases significantly with the CRISPR array length. Moreover, we found the *Campylobacter* bacteriophage DA10 is targeted by the CRISPR-*Cas* system of the majority of *C. jejuni* strains with a preserved CRISPR array. Moreover, this study identified 38 novel CTs and 20 novel spacer sequences in the CRISPR array, and demonstrates that GBS-related *C. jejuni* strains contain unique CT patterns compared to enteritis- and healthy control-related strains.

The type II CRISPR-Cas system has previously been reported to be involved in endogenous gene expression and gene regulation, respond to envelope stress and regulate other physiological processes in bacteria [25,35]. The ability of the Cas9 endonuclease to regulate the virulence genes of bacteria containing the type II CRISPR-Cas system were described in experimental studies of *C. jejuni* [15,36], *Francisella novicida* [37] and *Neisseria meningitides* [38]. *C. jejuni* strains harbouring the *Cas9* gene were found to be more virulent than strains that do not harbour *Cas9* or contain a degenerated *Cas9* gene [15]. Recently, Dugar *et al*. reported that *C. jejuni Cas9* can regulate gene expression by targeting endogenous mRNA [39]. However, the relationship between CRISPR array preservation and bacterial virulence or pathogenesis had not previously been assessed. This study provides the first evidence that preservation of the CRISPR array in *C. jejuni* is associated with the development of GBS, which suggests a preserved CRISPR array may contribute to virulence or immunogenicity by controlling the expression of bacterial genes that increase the risk of developing GBS.

The CRISPR array length depends on the number of spacers acquired by the bacteria (Fig. 4). In this study, we observed longer *C. jejuni* CRISPR arrays were associated with a higher probability of developing GBS. This observation can potentially be explained by the findings of Martynov *et al*., who reported that bacteria maintain the optimum numbers of spacers to gain an evolutionary benefit [40]. Additionally, Levin's mathematical model predicted that a preserved CRISPR array provides a competitive advantage to bacteria by facilitating long-term co-evolutionary arms races between phage and bacteria [41]. This study supports the mathematical prediction that bacteria prefer to maintain an optimum CRISPR array length.

We also confirmed that CRISPR array-based typing had more discriminatory power and sensitivity than PCR-based subtyping due to the highly polymorphic CRISPR arrays of enteritis- and healthy control-related *C. jejuni* strains; thus, CRISPR array-based typing may represent a superior genotyping method [42]. CRISPR-

her bacteria were previously reported [45–47]. Cardenas *et al*.

and Yeh *et al.* found CRISPR typing of *C. jejuni* had sufficient discriminatory power (SID) to be considered a useful genotyping method [28,44]. Our study provides additional evidence to suggest that CRISPR typing could represent a useful molecular detection tool for epidemiological source identification during outbreaks of GBS or *C. jejuni*-induced enteritis.

A major part of the genome of the recently discovered *Campylobacter* phage DA10 was found to be attacked by the *C. jejuni* CRISPR array [48,49]. Similarly, most of our CRISPR array-containing *C. jejuni* strains acquired spacers from the *Campylobacter* phage DA10.

This study has several limitations. Even though we used PCR-based CRISPR typing, the discriminatory power of CRISPR typing was not compared with traditional genotyping or serotyping methods such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) or *flaA* serotyping. Additionally, the sample size was relatively small and the significance of the association between *C. jejuni* CRISPR array preservation and the development of GBS needs to be verified by an experimental study.

Overall, this study reveals that preservation of the CRISPR array in *C. jejuni* may provide an additional evolutionary benefit during the immunopathogenesis of GBS, which establishes a link between CRISPR array preservation and the risk of disease development. Further studies of larger, multicentric cohorts and genomic analysis of the entire *C. jejuni* CRISPR-Cas system using an experimental study design are required to confirm the role of the *C. jejuni* CRISPR array in bacterial virulence and the pathogenesis of GBS.

Methods

Study population

We isolated 30 *C. jejuni* strains from the stools specimen of patients with GBS and 52 *C. jejuni* strains from ethnically matched healthy controls as part of a prospective case-control study at the icddr,b. In addition, we included 60 historical *C. jejuni* strains from patients with enteritis in this study. All patients with GBS fulfilled the National Institute of Neurological Disorders and Stroke (NINDS) diagnostic criteria for GBS [50]. This study was reviewed and approved by the Ethics Committee of the icddr,b, Dhaka, Bangladesh, and all participants provided written informed consent prior sample collection. All methods were carried out in accordance with relevant guidelines and regulations.

Bacterial strains and growth

Faecal samples were collected at the time of enrolment of each participant. *C. jejuni* were cultured on blood agar plates containing 5% sheep blood at 37°C for 48 h under microaerobic conditions (6% O_2, 7% CO_2, 80% N_2, 7% H_2) using an Anoxomat system (Anoxomat™ Mart II, Drachten, The Netherlands). *C. jejuni* colonies were identified by oxidase, catalase and hippurate hydrolysis tests and confirmed by *C. jejuni* species-specific polymerase chain reaction (PCR) using the forward primer 5 $-GA\forall GACGCT\top CGCTGCTG-3$ and reverse primer 5-$AGCTAGC\top CGCATAATAACTTG$-3 [51].

All confirmed strains were stored at -80°C in brain heart infusion broth containing 15% glycerol until analysis [30].

Extraction of bacterial genomic DNA

Bacterial DNA was extracted from colonies collected from the blood agar plates using Qiagen Genomic DNA purification kits, according to the manufacturer's instructions (Qiagen, Venlo, the Netherlands). DNA samples were stored at -20°C prior CRISPR typing.

Determination of C. jejuni CRISPR-Cas components

The components of the *C. jejuni* CRISPR-Cas system were detected by PCR. The first forward primer for the CRISPR array, F1 (see Supplementary Table S3 online), was designed to target the left flanking region (leader sequence) of the CRISPR array to detect confirmed or true CRISPR array loci [28]. The second forward primer for the CRISPR array, F2 (see Supplementary Table S3 online), was designed to overlap the first 13 nucleotides (Fig. 4) of the most common consensus direct repeat (DR) of *C. jejuni* in the CRISPRdb [22]. The reverse primer for the CRISPR array was obtained from previously published studies [42,43]. The NCBI Basic Local Alignment Search Tool (BLAST), MUSCLE and OligoAnalyzer 3.1 were used to design the primers to detect the *Cas* genes [36].

The amplification reactions were performed in 25 µL PCR master mixes containing ~ 10 ng of *C. jejuni* chromosomal DNA, 0.2 µL of each primer, 4 µL of 5× PCR buffer, 1.6 µL of 25 mM $MgCl_2$ and 0.2 µL *Taq* DNA polymerase enzyme on MJ Research PTC-200 ThermalCycler (Marshall Scientific, Hampton, NH, USA). The reaction conditions were initial denaturation at 95°C for 10 min, followed by 35 cycles of 45 sec denaturation at 94°C, 30 sec annealing at different temperature for *Cas 1, Cas2, Cas9* and the CRISPR array showed in Supplementary Table S3 online and 40 sec extension at 72°C, and a final extension step at 72°C for 10 min. PCR products were visualized by electrophoresis on 1.5% agarose gels using a Molecular Imager® Gel Doc™ XR + system (Bio-Rad Laboratories Inc., Hercules, CA, USA).

CRISPR-based genotyping

Initial subtyping of the *C. jejuni* isolates was based on the CRISPR array length determined by PCR screening [52,53]. The CRISPR array length was calculated by subtracting 155 from the size of the PCR product amplified using the F2 primer (Fig. 4). The number of spacers and DRs were determined from the size of the PCR products. The repeat number is always one greater than the spacer number, as each spacer is flanked by two DRs [54]. *C. jejuni* strains containing at least one spacer sequence in their CRISPR array were classified as having a preserved CRISPR array; otherwise, the strain was termed degenerate [41].

The PCR products amplified from 20 GBS-, 29 enteritis- and 21 healthy control-related CRISPR array preserving *C. jejuni* isolates were purified using the ExoSAP-IT® PCR Product Cleanup Reagent (Affymetrix, Cleaveland, OH, USA) at 37°C for 15 min, followed by 15 min inactivation at 80°C. Cycle sequencing was carried out using the BigDye Terminator Kit (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and the final purified products were loaded onto an ABI 3500xL Automated Genetic Analyzer (Perkin-Elmer

Applied Biosystems) to determine the spacer polymorphism-based CRISPR type (CT). Each unique spacer sequence was given an allele number based on its chronology of discovery (see Supplementary Table S2 online). Each unique spacer arrangement was annotated as a unique CRISPR type (CT). The sequences of the *C. jejuni* CRISPR arrays were deposited in GenBank under accession numbers MW561358 to MW561427.

CRISPR array sequence analysis

CRISPR array sequences were detected using the web-based software CRISPRFinder (https://crispr.i2bc.paris-saclay.fr/Server/) [55] and queried using the web tool CRISPRTarget (http://crispr.otago.ac.nz/CRISPRTarget/crispr_analysis.html) to detect target proto-spacers in the spacer sequences using the GenBank-Phage, Refseq-Plasmid and Refseq-Viral databases [56]. The CRISPR array sequences were also screened using the Nucleotide BLAST web tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine novel CTs [57]. Phylogenetic analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean) function of MEGA X software [58]. Sequence logo was created using the web tool WebLogo (https://weblogo.berkeley.edu/logo.cgi) [59].

Statistical analysis

The discriminatory power of the various methods of CRISPR typing were compared by calculating Simpson's index of diversity (SID) with an online tool (http://www.comparingpartitions.info/?link=Tool) [60]. Association studies were conducted using the Chi-squared test and binary logistic regression. *P*-values < 0.05 were considered significant for associations and the fitness of regression model. Statistical analyses were performed using MedCalc software (version 19.6.4, MedCalc Software Ltd, Ostend, Belgium) and SPSS (version 20, SPSS Inc., Chicago, IL, USA) software.

Declarations

Acknowledgment

This research activity was funded by the Fogarty International Center, National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health (NIH), USA, under Award Number K43TW011447 and icddr,b. icddr,b acknowledges with gratitude the commitment of the Government of Bangladesh to its research efforts and gratefully acknowledges the unrestricted support provided by the Governments of the People's Republic of Bangladesh, Canada, Sweden, and the UK. We thankfully acknowledge the neurologists who referred their patients to this study.

Author contributions statement

Z.I., M.I.R. and Y.H. conceptualized and designed the study; Y.H. and A.D. contributed to data acquisition. Y.H., A.D., I.J. and S.H. analysed and interpreted the data; A.A. submitted the sequencing data to GenBank; Y.H. drew schematic illustration of the CRISPR array; Y.H. wrote the first draft of the manuscript along with

I.J. and S.H., which was critically reviewed by all other co-authors. The entire study was performed under Z.I. supervision. All authors gave their consent to publish the study.

Additional information

Competing interest

The authors declare no competing interest.

Availability of data

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. The sequencing data are available from the National Center for Biotechnology Information (NCBI) GenBank under the accession number MW561358 to MW561427.

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Figures

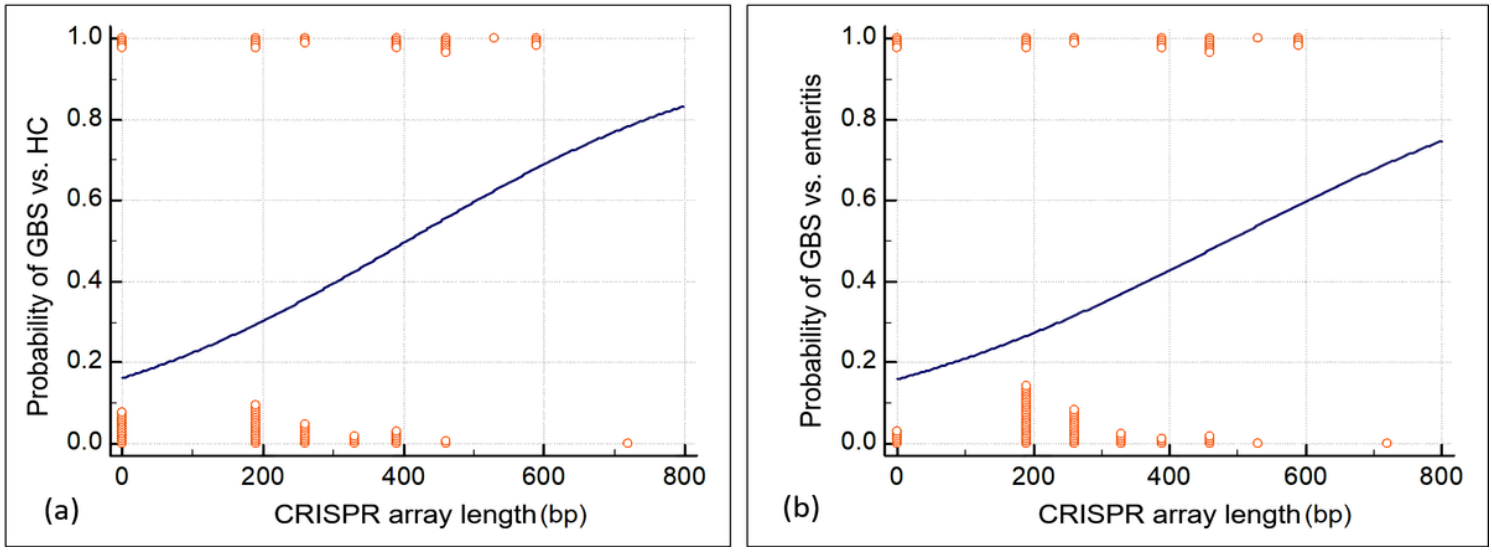

Figure 1

(a) Binary logistic regression curve (prepared using MedCalc software) predicting the probability of developing GBS following C. jejuni infection based on the C. jejuni CRISPR array length compared to healthy controls. (b) Binary logistic regression curve predicting the probability of developing GBS following C. jejuni infection based on the C. jejuni CRISPR array length compared to the probability of developing enteritis

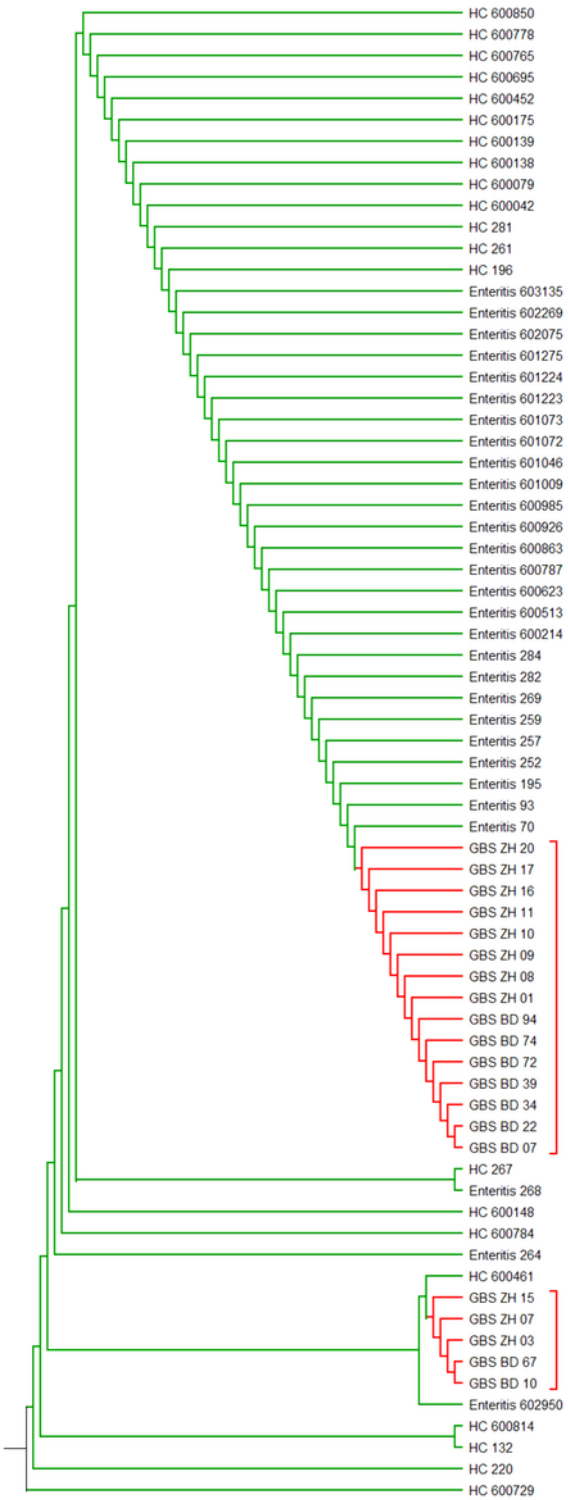

Figure 2

UPGMA dendrogram (prepared using MEGA X software) of C. jejuni CRISPR arrays from 20 strains isolated from patients with GBS, 29 strains isolated from patients with enteritis and 21 strains isolated from healthy controls. Clusters of GBS-related strains are indicated by red branches; clusters of enteritis and healthy control-related strains are indicated by green branches

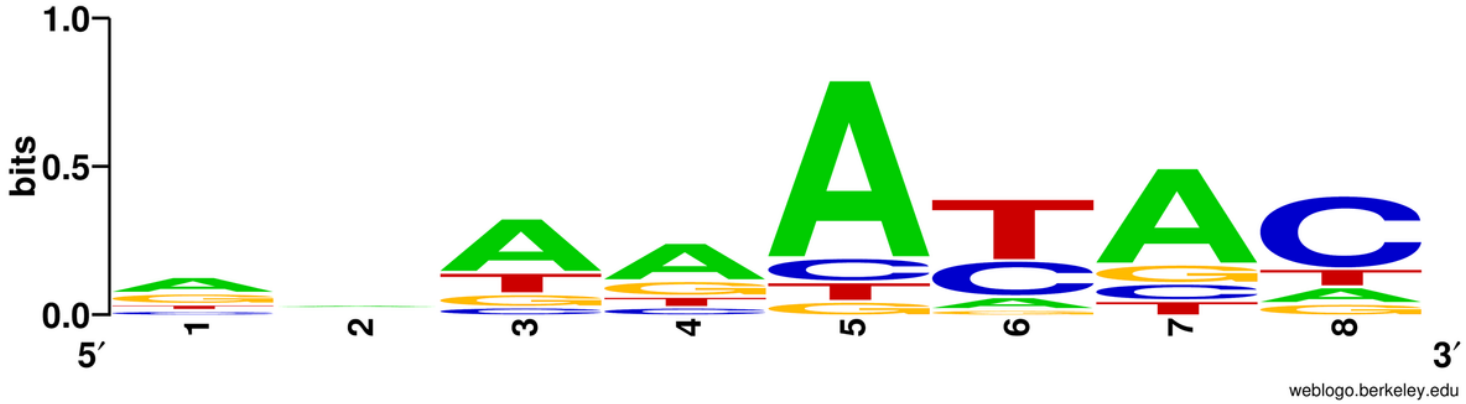

Figure 3

Sequence logo of the 3$downstreampro \rightarrow$ - $spacersequences, \in dicat \in g$3-NNNNAYAC-5` is the putative proto-spacer adjacent motif (PAM) of the Cas9 endonuclease in all 70 CRISPR array-containing C. jejuni strains isolated from patients with GBS, enteritis and healthy control

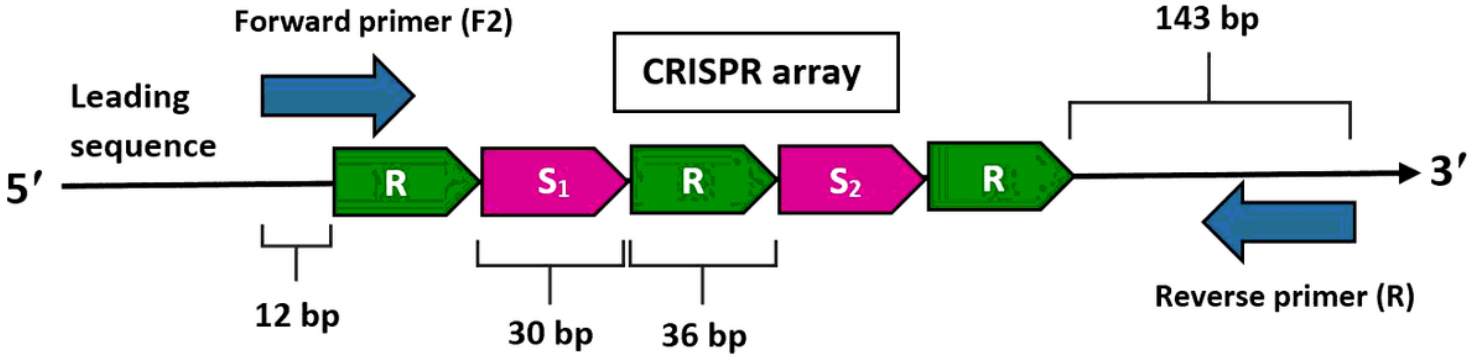

Figure 4

Schematic illustration of the CRISPR array structure and the targeting primers. R indicates the consensus direct repeat (DR) of the C. jejuni CRISPR array (36 bp); S indicates a spacer sequence (30 bp), which is always flanked by two DRs. The C. jejuni DR-specific forward primer (F2) is 25 bases long and overlaps the first 13 bases of the DR

Supplementary Files

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