International Journal of Medical Microbiology 307 (2017) 233–240

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Accessory genetic content in *Campylobacter jejuni* ST21CC isolates from feces and blood

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ARTICLE INFO

Keywords:
Campylobacter
Invasive infection
Comparative genomics
Invasion assays
DNA recombination

ABSTRACT

Campylobacter jejuni is an important foodborne pathogen and the most commonly reported bacterial cause of gastroenteritis. *C. jejuni* is occasionally found in blood, although mechanisms important for invasiveness have remained unclear. *C. jejuni* is divided into many different lineages, of which the ST21 clonal complex (CC) is widely distributed. Here, we performed comparative genomic and *in vitro* analyses on 17*C. jejuni* ST21CC strains derived from human blood and feces in order to identify features associated with isolation site. The ST21CC lineage is divided into two large groups; centered around ST-21 and ST-50. Our clinical strains, typed as ST-50, showed further microevolution into two distinct clusters. These clusters were distinguished by major differences in their capsule loci and the distribution of accessory genetic content, including *C. jejuni* integrated elements (CJIEs) and plasmids. Accessory genetic content was more common among fecal than blood strains, whereas blood strains contained a hybrid capsule locus which partially consisted of *C. jejuni* subsp. *doylei*-like content. *In vitro* infection assays with human colon cell lines did not show significant differences in adherence and invasion between the blood and fecal strains. Our results showed that CJIEs and plasmid derived genetic material were less common among blood isolates than fecal isolates; in contrast, hybrid capsule loci, especially those containing *C. jejuni* subsp. *doylei*-like gene content, were found among many isolates derived from blood. The role of these findings requires more detailed investigation.

1. Introduction

Campylobacter jejuni is a major cause of gastrointestinal disease and an important antecedent for severe post-infectious complications such as the Guillain-Barré syndrome (Anon., 2015; Nyati and Nyati, 2013). Bacteremia has been estimated to occur in approximately 1% of the patients infected with *C. jejuni* (Louwen et al., 2012; Feodoroff et al., 2011), but the mechanisms of invasive infection have remained elusive.

Whole genome sequencing studies have facilitated the understanding of the genome biology of *C. jejuni* and other gastrointestinal pathogens and a large number of sequences for comprehensive comparative analyses are now available in public repositories. *C. jejuni* genomes are rather small compared to those of other gastrointestinal pathogens, with sizes ranging between 1.60 and 1.90 Mbp (Parkhill et al., 2000; Fouts et al., 2005). Characteristic of most *C. jejuni* genomes is the presence of hypervariable regions, which can differ greatly between different strains. Cell membrane structure proteins, such as those belonging to the lipooligosaccharide and capsule loci, can be found in such genomic regions (Parkhill et al., 2000). Some strains also contain *C. jejuni* integrated elements (CJIEs), which are remnants of prophages and genomic islands (Fouts et al., 2005) and often contain restriction endonucleases, which affect the ability of strains to take up foreign DNA (Fouts et al., 2005; Gaasbeek et al., 2009; Gaasbeek et al., 2010). Several studies have shown that CJIEs seem randomly distributed among different *C. jejuni* strains, although association with several different lineages has occurred (Parker et al., 2006; Quiñones et al., 2008; Clark et al., 2012; Skarp et al., 2015; Kivistö et al., 2014). In addition, several plasmids such as pTet and pVir have been characterized, which contain genes coding for products involved in antimicrobial resistance and conjugative transfer (Bacon et al., 2000). Also, *C. jejuni* has been shown to contain different types of restriction modification systems, consisting of a restriction endonuclease and a methylase, which allow for varying methylation patterns and possibly different disease outcomes (Zautner et al., 2015; Mou et al., 2014).

An interesting aspect of *C. jejuni* population biology is the association of particular lineages with certain sources or reservoirs and their global occurrence. Generalist lineages are found from a wide variety of sources and reservoirs worldwide, whereas specialist lineages can be

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http://dx.doi.org/10.1016/j.ijmm.2017.04.001
Received 27 November 2016; Received in revised form 30 January 2017; Accepted 2 April 2017

considered to be limited to a specific source or geographical region (de Haan et al., 2010a; Gripp et al., 2011; McTavish et al., 2008; Ogden et al., 2009; Sheppard et al., 2014). ST677CC can be considered a specialist lineage for which no specific source or reservoir has definitively been described so far and which has been overrepresented among isolates with a Finnish origin (Kärenlampi et al., 2007; de Haan et al., 2010b; Feodoroff et al., 2013). In contrast, ST21CC is considered a generalist lineage due to its ubiquitous and global distribution (Gripp et al., 2011; Sheppard et al., 2014; Sopwith et al., 2008) and often belongs to the top three most common lineages in large molecular epidemiological *C. jejuni* studies (de Haan et al., 2010a; Gripp et al., 2011; Sheppard et al., 2009)

Previously, we used Illumina sequencing to study the genomes of *C. jejuni* ST677CC; a highly abundant lineage among isolates detected from human blood and feces in patients with domestically acquired disease in Finland (Feodoroff et al., 2013; Skarp et al., 2015). In this particular lineage, an abundance of restriction-modification (RM) systems and orphan restriction enzymes was observed (Skarp et al., 2015), but virulence properties, such as the *cdtABC* operon (coding for the only known *Campylobacter* toxin CDT), and genes coding for sialylated lipooligosaccharide structures, previously suggested to be important in severe disease (Keo et al., 2011), were disrupted or absent (Skarp et al., 2015; Kivistö et al., 2014). However, an extended version of the HS4 capsule locus was described (Skarp et al., 2015; Kivistö et al., 2014), which included genes from *C. jejuni* subsp. *doylei*, a *Campylobacter* species suggested to be associated with bacteremia (Morey, 1996).

Many of the whole genome sequenced *C. jejuni* strains available to date have been derived from human or animal feces and environmental reservoirs. However, few studies have investigated the genomes of *C. jejuni* derived from other clinical samples, such as blood. This could be valuable in order to determine bacterial genomic content that could be involved in the development of invasive infections. In this study, we used comparative genomic analyses to characterize blood and fecal *C. jejuni* isolates belonging to generalist lineage ST21CC. Whole genome sequences for ST21CC available from public repositories were included for global comparative genomics. Evolutionary and recombination analyses were used to visualize the phylogenetic structure of the lineage.

2. Materials and methods

2.1. Bacterial isolates, DNA extraction and whole genome sequencing

Bacterial strains were cultivated and DNA was extracted for a total of 17 *C. jejuni* strains (Supplementary Table S1 in the online version at DOI: 10.1016/j.ijmm.2017.04.001), which were derived from human blood and feces as described earlier (Skarp et al., 2015). Illumina sequencing and genome assembly were performed as described before (Skarp et al., 2015). Briefly, 50 ng of extracted DNA was processed using the Nextera XT DNA Sample Preparation and Indexing Kits (Illumina, San Diego, CA, USA) for paired-end sequencing on a MiSeq Desktop Sequencer (Illumina). The reads were assembled *de novo* using SPAdes 3.0.0 and a5 miseq pipeline (Bankevich et al., 2012; Coil et al., 2015) and SSPACE v3 (Boetzer et al., 2011) was used to generate scaffolds from assembly contigs. The genomes were initially annotated by RAST (Aziz et al., 2008) and final annotation was done using the NCBI Prokaryotic Genome Annotation Pipeline (NCBI_PGAP). Read mapping was performed using appropriate reference sequences in Geneious.

2.2. Genomic analyses of ST21CC

For phylogenomic analysis, 17 ST21CC genomes characterized here and 19 publicly available ST21CC genomes (Supplementary Table S1 in the online version at DOI: 10.1016/j.ijmm.2017.04.001) were compared using the Gegenees software tool (Ågren et al., 2012). The comparison was performed using BlastN with 500 bp fragment sizes and a sliding window of 500 bp. Threshold levels were set at 5% of the maximum score values. The pair-wise similarities were exported in Nexus file format and visualized in SplitsTree4 (Huson and Bryant, 2006; Kloepper and Huson, 2008). Parsnp (Treangen et al., 2014) (http://harvest.readthedocs.org/en/latest/content/parsnp.html) was additionally used to reconstruct the core genome phylogeny of the ST21CC lineage. The options random reference, recombination detection and inclusion of all genomes were chosen and the tree file was visualized using MEGA6 (Tamura et al., 2013). The Blast Ring Image Generator (BRIG) was used to visualize genome comparisons (Alikhan et al., 2011).

2.3. In vitro cell adhesion and invasion assays

HT29 cells were grown in Dulbecco's modified Eagle's medium supplemented with 2 mM L-Glutamin, 10% FCS, 100 U/ml penicillin and 100 µg/ml streptomycin. The day before infection, a fixed number of cells were seeded in media without penicillin and streptomycin. One hour before infection, the media was replaced with infection media containing 1% FCS. Bacterial cultures, grown in Brucella broth (Becton, Dickinson and Company, Franklin Lakes, USA) at 42 °C for 18 h in a microaerobic atmosphere (CampyGen, Oxoid Ltd, Basingstoke, England), were centrifuged and the concentration was adjusted in infection media. Bacteria were added to cells at a MOI of 100. After 8 h, the cell culture media was collected and immediately frozen in −80 °C. Cells were washed 4 times with large volumes of PBS and thereafter lysed in 20 mM Tris, pH 7.5, 150 mM NaCl, 0.15% Triton X-100 to release adhered and invaded bacteria.

2.4. Quantification of adhered and invaded bacteria using real time qPCR

The lysate was diluted and was used together with diluted starting cultures in real time qPCR to determine the percentage of recovered bacteria. qPCR was run in BioRad CFX96 using DyNAmo HS SYBR green mix (Thermo Fisher Scientific) according to the manufacturer's protocol, and using primers GCGTAGGCGGATTATCAAGTC and CGGATTTTACCCCTACACCAC amplifying the 16S rRNA gene of *Campylobacter*.

2.5. Host cell IL-8 expression

IL-8 levels in the cell culture media were determined using the IL-8 ELISA Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Media was diluted 10 times prior to the assay. A standard of known concentration (included in the kit) was used to assess variations between infections. Results were expressed as fold increase over uninfected (mock) cells.

2.6. In silico analysis of a unique ST-50 Type III restriction modification system

The orthAgogue output was queried for accessory gene content and the phylogeny of a Type III restriction-modification (RM) system was reconstructed using the M subunit. Nucleotide sequences for Type III RM M subunits of other *C. jejuni* and *Campylobacter* as well as *Helicobacter* species were retrieved from public repositories (Supplementary Table S2 in the online version at DOI: 10.1016/j.ijmm.2017.04.001) and aligned using MAFFT (Katoh and Standley, 2014). The alignment was cleaned up using Gblocks (Castresana, 2000) with the less stringent options at the Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) and the phylogeny was reconstructed using MrBayes (Ronquist et al., 2012) with the settings nst = 6 and rates = invgamma. A total of 30,000 Markov chain Monte Carlo (MCMC) generations were run to obtain a standard deviation of

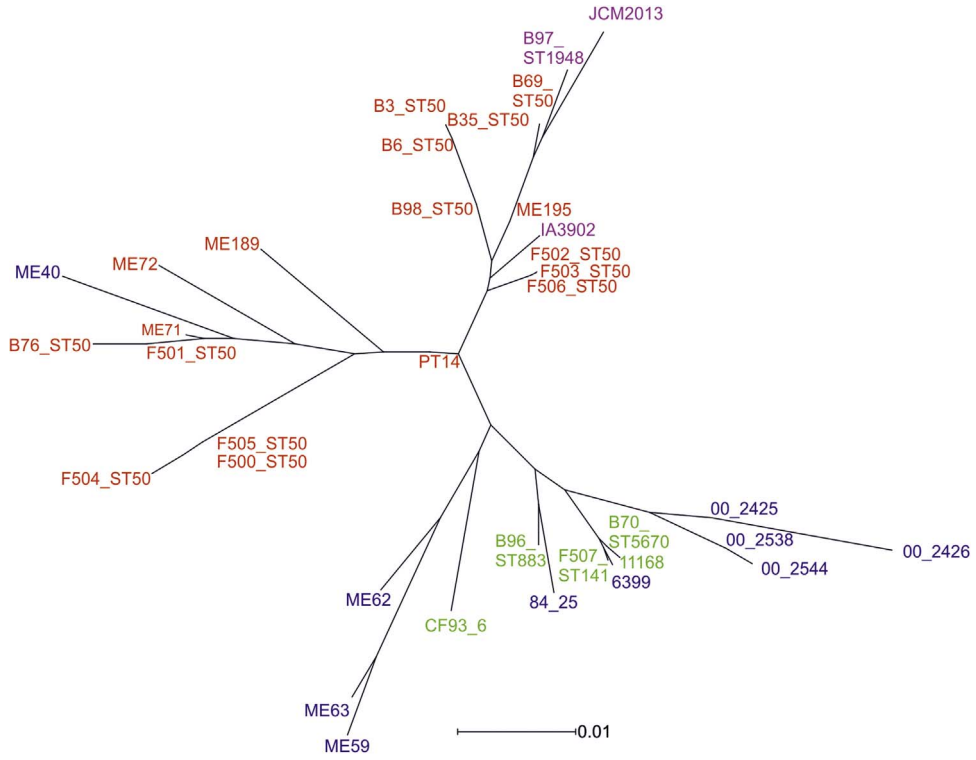

Fig. 1. Phylogenomic overview of the ST21CC lineage. The blood strains are assigned the prefix 'B' and the fecal strains are assigned the prefix 'F' and strains described by Sheppard et al., (Sheppard et al., 2013) have the prefix 'ME'. Strains assigned ST-50 are shaded red and strains belonging to a different ST in the ST-50 cluster are shaded purple. ST-21 strains are shaded blue, other STs within this cluster are shaded green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the split frequencies below 0.01.

3. Results and discussion

3.1. Phylogenomic structure of ST21CC

A fragmented alignment approach and subsequent phylogenomic reconstruction using Gegenees and SplitsTree showed that the ST21CC lineage divided into two large clusters; one containing ST-21 and related STs (ST-21 group) and one composed of ST-50 and related STs, except for one ST-21 strain (designated ME40 (Sheppard et al., 2013)), which was grouped within the ST-50 and ST-50 like cluster (Fig. 1). Only three of the 17 Finnish strains described in this study belonged to the ST-21 group; B70 (ST-5670), B96 (ST-883) and F507 (ST-141), and the remainder of our strains (all ST-50, except for B97 typed as ST-1948) belonged to the ST-50 cluster. We noted a tendency for the Finnish ST-50 strains to partially cluster according to their original isolation site (i.e. blood vs feces) in the core genome analysis, which showed a further stark divide between the ST-21 and ST-50 groups and within the ST-50 cluster (Fig. 2). In contrast to the ST-50 group clustering in the whole genome analysis, the ST-21 strain designated as ME40 (Sheppard et al., 2013) and strain IA3902 (Wu et al., 2013) clustered into the ST-21 group in the core genome analysis. This indicates that these strains are more closely related to ST-21 and ST-21 like strains, but as genetic exchange between the ST-21 and ST-50 groups occurs, this subsequently has obscured the phylogenetic signal in pan genomic analysis of even closely related lineages. The two clusters seen among the ST-50 strains were further analyzed for major differences, which were found in distribution of CJIEs, restriction modification (RM) systems, capsule loci and presence of plasmid DNA.

3.2. CJIEs

Of our 17 clinical strains belonging to ST21CC, none of the three strains in the ST-21 group had any of the five CJIEs described to date (Fouts et al., 2005; Skarp et al., 2015). However, the majority of strains typed as ST-50 (9/13; 69%) had one or two CJIE (Table 1) and most of these CJIE-positive strains were derived from fecal material (7/9; 78%). This contrasts an earlier finding of ours in the ST677CC lineage, in which all strains contained an integrated element (CJIE1 prophage), regardless of the isolation site (Skarp et al., 2015). It also contrasts the results of the presence of CJIEs in the other (already published) genomes used in the analysis, as these show that the ST-21 and ST-21 alike strains more often contain CJIEs than the ST-50 and ST-50 alike strains (Supplementary Table S3 in the online version at DOI: 10.1016/j.ijmm.2017.04.001). This could be due to a limited number of strains analyzed and a similar study with larger datasets is required to confirm our findings. Although little evidence is currently available for the added benefit of CJIE1 in human infections, an earlier study showed that CJIE1-positive strains were on average more adherent and invasive *in vitro* than CJIE1-negative strains (Clark et al., 2012). We performed *in vitro* cell adhesion and invasion assays with our strains. Recovered bacteria from infected HT29 colon cancer cells ranged between 0.2 and 3%, which was less than with the *C. jejuni* NCTC 11168 and 81-176 reference strains (Fig. 3A). There was no significant difference between the blood and fecal strains (Fig. 3B). Neither was there any difference in the ability of the strains to evoke an IL-8 response in these cells (Fig. 3C and D). Furthermore, no direct correlation between adhesion and invasion potential and CJIE or plasmid status was seen (data not shown). Also, little difference was seen between the CJIE-positive and CJIE-negative strains in our study (data not shown). This discrepancy with the study by Clark et al. (2012) can be due to the use of different cell lines and infection times, although our recovery percentages were similar to theirs. Moreover, we did have more CJIE-negative strains in our study here, which may nuance findings from studies with smaller number of such strains.

Induction of the prophage during gut passage may result in jump-outs and reintegration at a different site (Clark et al., 2014). In the present study, different integration sites were found among our ST-50 CJIE-positive strains; whereas for the ST677CC lineage, CJIE1 is stably

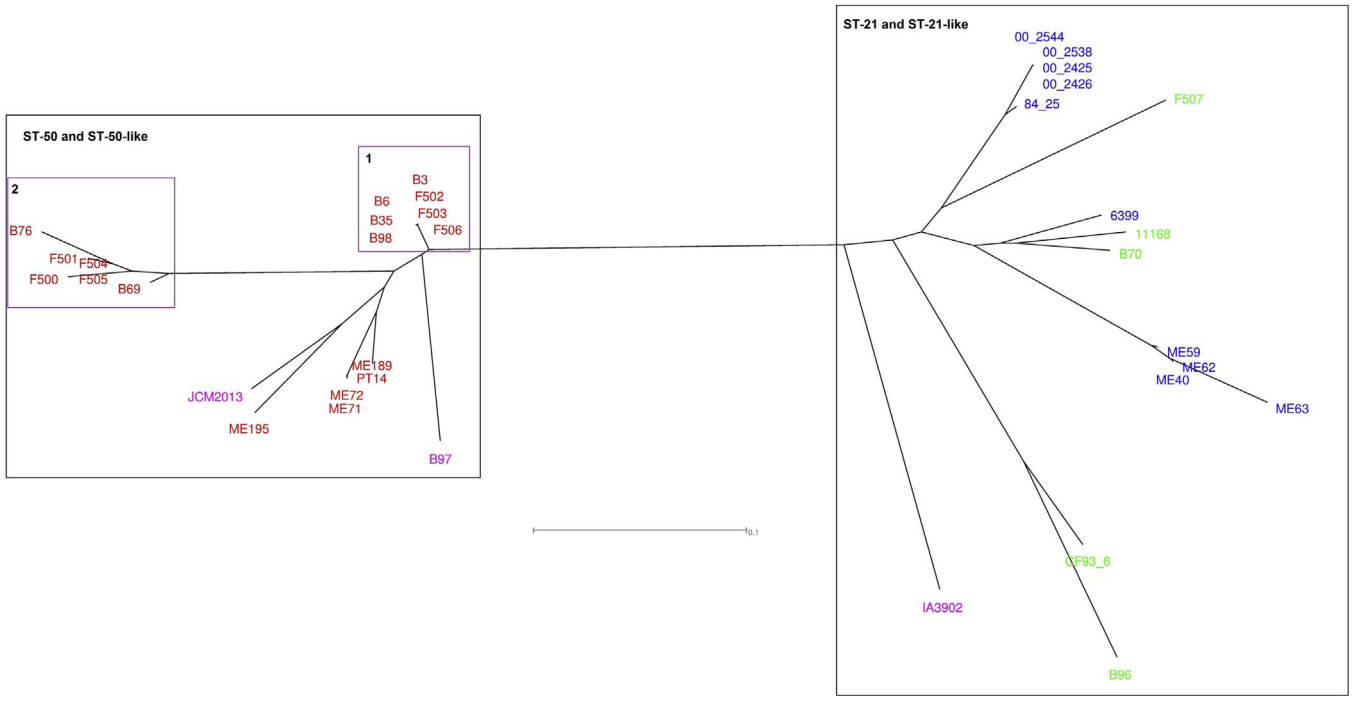

Fig. 2. Core genome analysis of the ST21CC lineage by Parsnp. The blood strains are assigned the prefix 'B' and the fecal strains are assigned the prefix 'F' and strains described by Sheppard et al., (Sheppard et al., 2013) have the prefix 'ME'. Strains assigned ST-50 are shaded red and ST-50-like strains are shaded purple; this includes IA3902, which clustered with ST-50 in the phylogenomic analysis. ST-21 strains are shaded blue and ST-21-like strains are shaded green. ST-50 clusters are numbered. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Presence of *C. jejuni* integrated elements (CJIE) and plasmids in Finnish ST21CC Strains isolated from blood (B) and feces (F)

Strain	ST	ST-50 core cluster	Genome size (Mbp)	CJIE1	CJIE4	Plasmid	ST-50 Type III RM	CPS HS1,8	CPS HS5/31,15
B3	50	1a	1.62				√	√	
B6	50	1b	1.62				√	√	
B35	50	1b	1.62				√	√	
B69	50	2	1.69	√		√[b]		√	
B70	5670[a]		1.61						
B76	50	2	1.77	√	√	√[b]		√	
B96	883[a]		1.63						
B97	1948[a]		1.63			√[c]			
B98	50	1b	1.62				√	√	
F500	50	2c	1.76	√	√	√[b]			√
F501	50	2	1.76	√	√	√[b]		√	
F502	50	1a	1.65	√		√[b]	√	√	
F503	50	1a	1.66	√		√[b]	√	√	
F504	50	2c	1.75	√	√	√[b]			√
F505	50	2c	1.68	√	√	√[b]			√
F506	50	1a	1.67	√		√[c]	√	√	
F507	141		1.62						

[a] ST-21 group.
[b] pCC42yr-like(Pearson et al., 2013).
[c] pTet-like (Bacon et al., 2000).

integrated downstream of the GTPase Era (Kivistö et al., 2014). Altogether, the presence of CJIE1 may not be crucial or beneficial for the bacterium during the course of an invasive infection. It is interesting to speculate that prophage induction, and simultaneous loss of the prophage, and invasion into the bloodstream could account for the major absence of CJIE1 in most of the ST-50 blood strains.

3.3. RM systems

C. jejuni can encode an abundant amount of different RM systems, which are either a stable part of its core genome or exist on small genomic islands or CJIEs. Both CJIE1 and CJIE4 have been shown to encode functional restriction endonucleases (REases) (Gaasbeek et al., 2009; Gaasbeek et al., 2010). In the current study, CJIE4 was exclusively detected among strains belonging to ST-50 cluster 2, whereas CJIE1 was detected in both clusters (Table 1). Additionally, ST-50 cluster 1 strains had a Type III RM system, which was not found in cluster 2 strains. We have earlier described a similar skew within the ST677CC lineage, between ST-677 and ST-794; some ST-677 strains carried CJIE5 containing two REases, whereas, all ST-794 strains had a novel Type III RM system (Skarp et al., 2015). Phylogenetic analysis indicated that the ST-794 Type III RM was closely related to one of the systems encoded by *Helicobacter acinonychis*, but not by *C. jejuni* RM1221 (Skarp et al., 2015). Similarly, in this study, phylogenetic

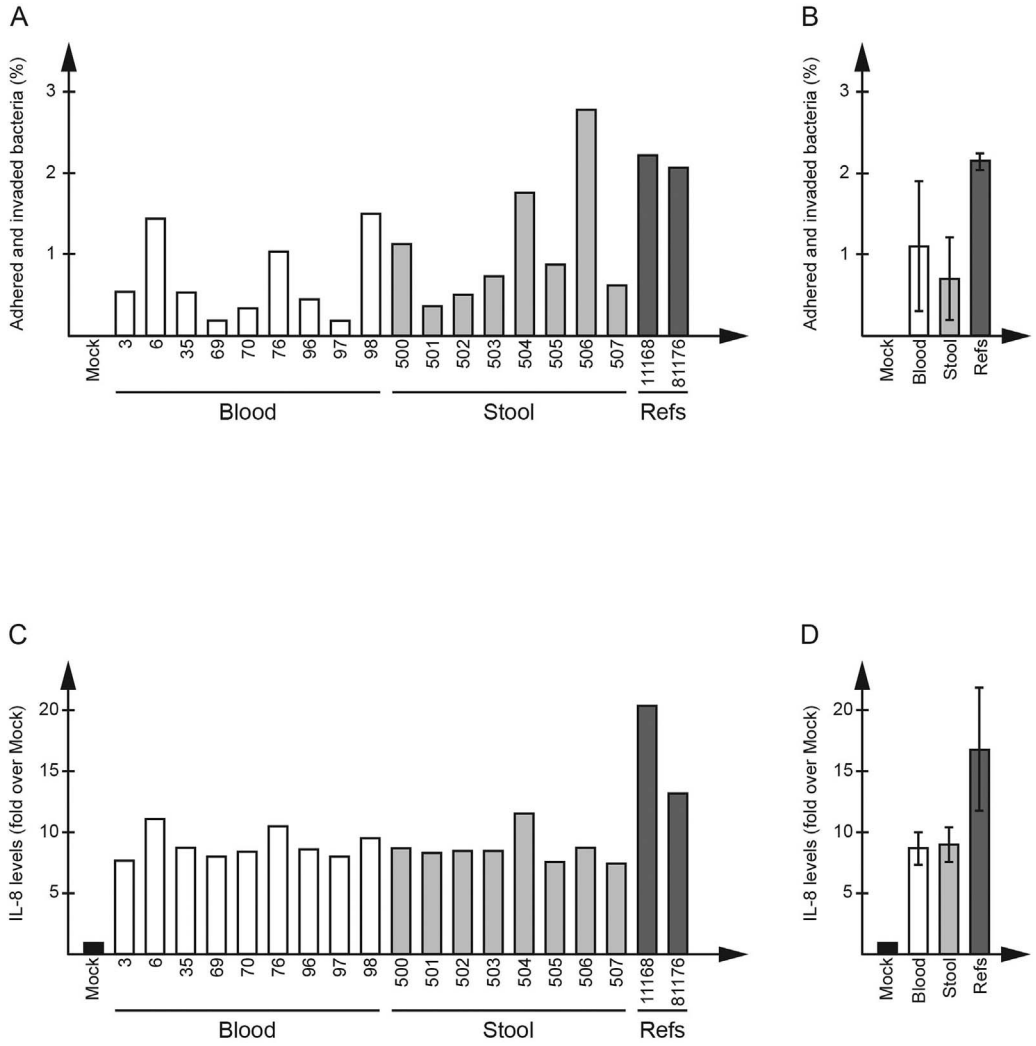

Fig. 3. *in vitro* adhesion and invasion assay. HT29 colon cancer cells were infected with each bacterial strain for 8 h using a MOI of 100. (A and B) Adhered and invaded bacteria were quantified using real-time qPCR and shown as a percentage of added bacteria. (C and D) Host cell IL-8 levels in the cell culture media were determined using ELISA. Graphs A and C show mean of each strain from two or three independent infections. Graphs B and D show means and standard deviations of blood, stool and reference strains together as groups.

analysis of the M subunit of ε-proteobacterial Type III RM systems showed that the ST-50 specific Type III M subunit was more closely related to one of the Type III M subunits of *C. upsaliensis* (HMPREF0429) rather than that of *C. jejuni* RM1221 (Fouts et al., 2005) (Supplementary Fig. S1 in the online version at DOI: 10.1016/j.ijmm.2017.04.001). Interestingly, this *C. upsaliensis* Type III RM system consists of a phase-variated methylation subunit (Fouts et al., 2005); whereas such phase-variation signal (such as a homopolymeric tract) was not detected for the ST-50 specific Type III RM, M subunit described here. Phase-variated RM systems could increase the fitness of the bacteria upon contact with different environments and may be beneficial in invasion of human cells (Srikhanta et al., 2011; Kwiatek et al., 2015). Nevertheless, the ST-50 Type III RM does not appear phase-variated and requires further characterization to understand its role in human infections. Finally, the various restriction mechanisms between the two ST-50 clusters may either be a consequence or a result of genetic isolation and genome evolution (Vasu and Nagaraja, 2013), which would be in line with the observed cluster formation of ST-50.

3.4. Capsular polysaccharide (CPS)

The CPS of *C. jejuni* is considered to play an important role in pathogenesis and earlier we have shown for ST677CC strains, over-represented among Finnish blood isolates, that their extended HS4 CPS locus includes *C. jejuni* subsp. *doylei* (Cjd)-like genes (Skarp et al., 2015; Kivistö et al., 2014). In this study, strikingly different capsule loci were found between the two ST-50 clusters; the CPS locus of strains belonging to cluster 1 and strains B69, B76 and F501 resembled the CPS locus of IA3902 (HS1,8); a highly pathogenic strain associated with abortion in sheep (Wu et al., 2013). Like the extended ST677CC HS4 locus, the HS1,8 CPS locus also contains Cjd-like genes. *C. jejuni* subsp *doylei* was shown to be common among patients suffering from *Campylobacter* bacteremia in Australia and South Africa (Morey 1996; Lastovica, 1996) and occurrence of Cjd-like genetic content in our *C. jejuni* isolated from blood may not be coincidental. The fecal strains F500, F504 and F505 had a unique CPS locus (Fig. 4); a hybrid of HS5/HS31 complex and HS15, earlier suggested to exist by Poly et al., (Poly et al., 2015). This CPS locus contained heptose biosynthesis genes and GDP-mannose 4,6-dehydratase (*wcbK*). *C. fetus* subsp. *fetus* Type B strains, in which *wcbK* is present, are serum sensitive, whereas Type A strains, with no *wcbK,* are serum resistant. The *wcbK* gene is also present in ST677CC and phase-variation of its expression has been associated with different degrees of serum resistance (Skarp et al., 2015). However, the *wcbK* gene belonging to ST-50 strains F500, F504 and F505 was not phase-variated. Based on prior observations with *C. fetus* subsp. *fetus* Type B and *C. jejuni* ST677CC we speculate that constitutive expression of *wcbK* in ST-50 will possibly lead to an overall lower resistance to human serum, but this remains to be tested. Altogether,

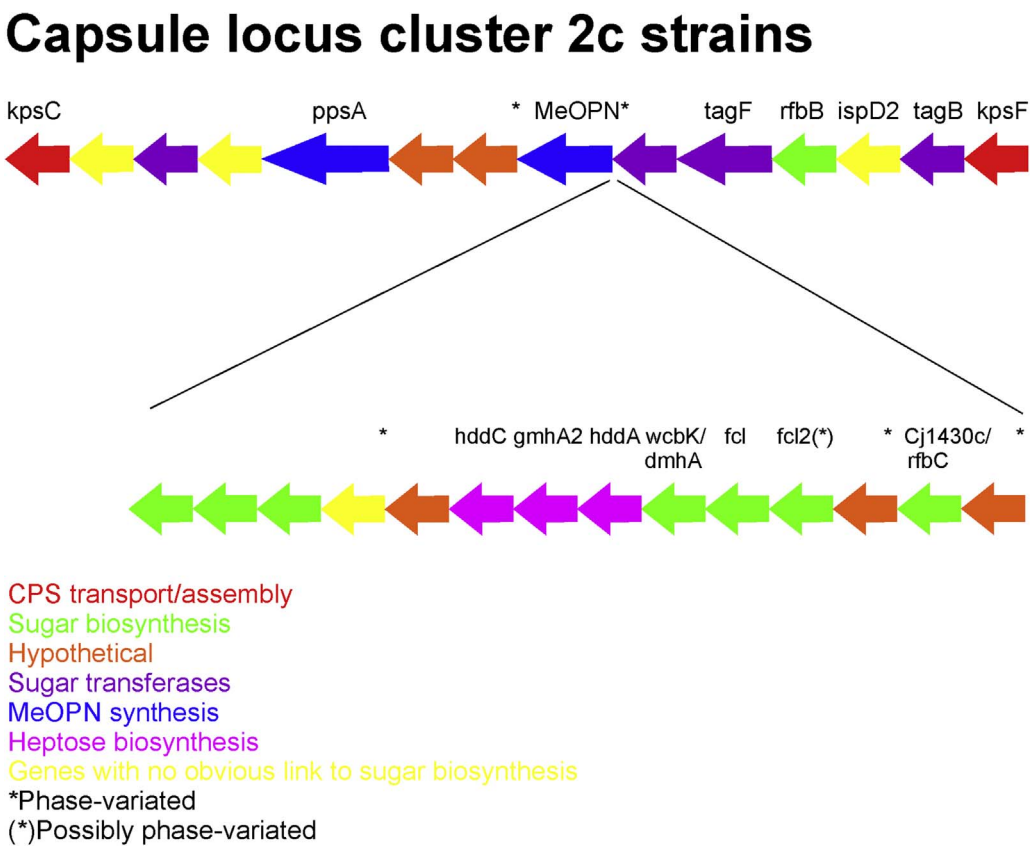

Fig. 4. Capsule locus of ST-50 strains F500, F504 and F505. The lower set of genes represents an insertion part of the HS15 capsule locus into the upper set of genes, which resembles capsule locus HS5/31.

our results here and earlier (Skarp et al., 2015) as well as results from others (Wu et al., 2013; Karlyshev et al., 2005) suggest that acquired capsule genes, especially those from *C. jejuni* subsp. *doylei*, might play a role in the development of severe *Campylobacter* infections.

3.5. Plasmids

Many of the ST-50 strains carried plasmids (Table 1); only strains B3, B6, B35 and B98 did not have any plasmids. Also, the three strains belonging to the ST-21 group did not have any plasmids. The plasmid sequences from strains B69, B76, F500, F501, F502, F503, F504 and F505 resembled the earlier sequenced plasmid pCC42yr (Pearson et al., 2013), with small random deletions in several of our strains (Supplementary Fig. S2 in the online version at DOI: 10.1016/j.ijmm.2017.04.001). A pTet-like plasmid was found for both strains B97 and F506, (Supplementary Fig. S3 in the online version at DOI: 10.1016/j.ijmm.2017.04.001) but the tetracycline resistance gene, *tetO*, was not present. None of the plasmids among our strains confers antimicrobial resistance, but genes for a Type IV secretion system, allowing for conjugative transfer, are present. The plasmids were present regardless of the numerous RM mechanisms encoded either chromosomally or on a CJIE, indicating that either recognition sequences were not present or the plasmid confers an as of yet unknown advantage to the bacterium. It is interesting to note though that most strains derived from blood did not have plasmids, which would suggest that plasmids do not encode important characteristics for invasive infection. Similar to the presence of CJIEs, which were also rare among ST-50 blood isolates, this would suggest further that ST-50 strains with less accessory genetic content may be able to survive longer in the bloodstream.

3.6. Other potential virulence determinants

Several previously described putative virulence features, such as genes for sialylated lipooligosaccharide (LOS) structures, CDT, and the CRISPR-Cas system were present in all our ST21CC strains, but either absent or highly fragmented in all ST677CC strains. Sialylated LOS has been associated with the post-infectious neuropathy Guillain-Barré syndrome (Godschalk et al., 2004) and CDT has been associated with cell cycle arrest and death (Smith and Bayles, 2006). The CRISPR-Cas system of *C. jejuni* is considered as an innate immune system, which prevents infection with bacteriophages, which may or may not work synergistically with sialylated LOS (Louwen and van Baarlen, 2013). It is unsurprising to find such diversity between unrelated lineages; but it also addresses uncertainty of the importance of such features in the development of different types of disease. Therefore, such characteristics can be dispensable to one lineage, but may prove invaluable to another one.

4. Conclusions

C. jejuni show a great potential in adaptation to different hosts and environments; this is also reflected in the highly diversified population structure comprised of many clonally unrelated lineages. Nevertheless, the pathogenic potential of many of these lineages, has not been affected by this diversification. In our study, acquired gene content such as CJIEs and plasmids, were more common among fecal than blood strains. It is tempting to speculate that *C. jejuni* strains with less acquired genetic content could be more successful in invasive infection. Simultaneously, the presence of Cjd-like acquired genetic content within the capsule locus might be beneficial in establishing an invasive infection and thus may be universally desirable to phylogenetically unrelated lineages.

Availability of data and materials

The whole genome sequences were deposited into the NCBI database (Bioproject PRJNA290425) and accession numbers for the strains can be found in Supplementary Table S1 in the online version at DOI: 10.1016/j.ijmm.2017.04.001.

Authors' contributions

CPAS performed the phylogenomic and comparative analyses and drafted the manuscript. OA preprocessed the data, performed genome assembly and submitted the genomes. RK performed the read mapping of capsule and plasmids. CJ performed the *in vitro* infection assays. HR conceived the idea, provided the resources and materials and drafted the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

We thank Anna Nilsson for DNA extraction of the strains for genome sequencing. This work was supported by the Swedish Research Council (grant 521-2011-3527) and Swedish Research Council FORMAS (grant 221-2012-1442). OA was supported by the Finnish Cultural Foundation, the Finnish Foundation for Cardiovascular Research, Ida Montin Foundation and Orion Research Foundation. No conflict of interest is declared.

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