**Summary**

*Campylobacter jejuni* is the most important reported cause for bacterial diarrhoea. The infection can be accompanied by fever and abdominal cramps and in rare cases the Guillain-Barré syndrome or reactive arthritis can develop as a post-infection complication. Several biological properties of *C. jejuni*, e. g. motility and chemotaxis, contribute to the biological fitness of the pathogen. For this, deficiencies in the function of these features clearly reduce the pathogenicity of *C. jejuni* without being a virulence factor per se.

Opposing to this, there are two essential requirements to determine the virulence of *C. jejuni* which represent the adherence to, and the invasion of host cells. Thereby, adherence, as a virulence factor, is mediated by many different bacterial-derived components like proteins but also by several oligo- and polysaccharide structures that are linked to surface proteins but also to the flagella.

In addition, several invasion-relevant features of *C. jejuni* have been detected so far. Whereas some of them are described functionally to modulate cytoskeleton arrangement of the host cell, others are only described as invasion relevant. Indeed, investigations with respect to the pathogenic potential of some adherence- and invasion-relevant components did not give identical results indicating that their relevance might depend on the interplay of the respective *C. jejuni* strains used in these studies with the corresponding host cells.

This review summarizes the *C. jejuni* components for adherence to and invasion of host cells together with their particular mode of action if known.

**Keywords:** *Campylobacter jejuni*, adherence, invasion

---

**Zusammenfassung**


Im Gegensatz hierzu stehen Faktoren des Erregers, die vor allem seine Virulenz bestimmen, wie z. B. die Adhärenz an und die Invasion in Wirtszellen. Die Adhärenzeigenschaften werden von verschiedenen bakteriellen Komponenten, wie beispielsweise Proteinen, aber auch Oligo- und Polysaccharidstrukturen, welche Protein-, aber auch Flagellar-gebunden sind, bestimmt. Zusätzlich sind verschiedene invasionsrelevante Komponenten von *C. jejuni* bekannt. Während von einigen dieser Invasionsfaktoren gezeigt werden konnte, dass sie die Zytoskelettstruktur der Wirtszelle verändern, sind andere lediglich als invasionsrelevant beschrieben. Allerdings sind die Forschungsergebnisse im Hinblick auf die Bedeu-
**Introduction**

*Campylobacter (C.) jejuni*, a spiral shaped, Gram-negative, microaerophilic bacterium, belongs to the delta epsilon group of proteobacteria. *C. jejuni* is flagellated leading to a corkscrew motility but does not possess the capability to form spores.

The first complete genome sequence of a *C. jejuni* strain (NCTC11168) was published in the year 2000. The circular genome has a size of 1.6 Mbp encoding 1,654 proteins and 54 stable RNA species (Parkhill et al., 2000). Meanwhile the complete genome sequences of 14 strains are available at the NCBI genome database. Compared to other intestinal pathogens like *Salmonella* spp., *C. jejuni* has a comparatively small genome and a rather low G+C content of approximately 30% (Gaskin et al., 2009). Plasmids encoding for antibiotic resistance or a type-IV-secretion system are described in several, but not all strains sequenced so far (Bacon et al., 2000; Batchelor et al., 2004; Louwen et al., 2006).

Unlike e.g. *Escherichia (E.) coli*, *C. jejuni* is incapable to ferment glucose due to the lack of the glycolytic enzyme phosphofructokinase. Only a livestock-adapted subgroup of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006). Due to the expression of a periplasmic *γ*-glutamyl-transpeptidase and a periplasmic asparaginase, a non-livestock adapted enzyme phosphofructokinase. Only a livestock-adapted group of *C. jejuni* strains is able to utilize L-fucose (Muraoka and Zhang, 2011; Zautner et al., 2012 a). Instead, *C. jejuni* is able to metabolize free amino and keto acids which have been generated by accompanying bacterial species in its ecological niche or directly from the respective host (Lee and Newell, 2006).

**Diminished pathogenicity by decreased biological fitness**

The pathogenicity of *C. jejuni* depends on its capability to interact and, subsequently, invade the respective host cell. Furthermore, other factors are essential to evolve the pathogenic potential, which are not virulence factors per se, but constitute a vital requirement for infection. In this context, most notably, flagellar motility and chemotaxis have to be mentioned. *C. jejuni* is adapted to reside within a viscous mucus layer which covers the gastric epithelial cells. This has led to the development of a powerful flagellar apparatus that confers motility to the bacterium and enables it to move with high velocities inside its particular microenvironment and to reach potential target cells. Consequently, motility of *C. jejuni* was shown to be a key factor for intestinal colonization and *C. jejuni* mutants with motility defects lose their competence to infect host cells (Morooka et al., 1985; Yao et al., 1994; Wassenaar and Blaser, 1999).

The chemoreceptors of *C. jejuni*, which detect variations of chemotaxin concentrations surrounding the bacterium, depict a direct control for the activity of the flagellar apparatus. Altogether ten different chemoreceptors and two aerotaxis genes have been described so far, whereby only three of the chemoreceptors have been characterized with respect to their chemotactic activity and chemorepellents (Hartley-Tassell et al., 2010; Tareen et al., 2014). These chemoreceptors are either membrane-bound or located in the cytoplasm and interact with a kinase, a response regulator and a coupling protein, respectively. A chemotactant bind-
ing to the chemoreceptor reduces the amount of phos-
phorylated response regulator, which finally diminishes its interaction with the flagellar apparatus. The lack of response regulator-flagellar switch interaction causes a counterclockwise flagellar rotation leading to bacterial swimming. Otherwise, the absence of a chemotactant promotes phosphorylation of the response regulator and binding to the flagellar switch. Consequently, the flagellum turns clockwise which is accompanied by tumbling and direction changes (Marchant et al., 2002; Zautner et al., 2012b; Lertsethtakarn et al., 2011). Due to the fact that the chemotactical system of C. jejuni directly governs the motility of the pathogen, mutations of chemoreceptors or accessory proteins strongly decreases the motility of C. jejuni and, thus, pathogenicity (Golden and Acheson; 2002; Hendrixson et al., 2001; Tareen et al., 2010; Bereswill et al., 2011).

**Adherence-mediating molecules of C. jejuni**

The adherence of C. jejuni to the epithelial cells of the intestine is an essential requirement prior to the process to invasion. During the last two decades several proteins, but also carbohydrates have been characterized that contribute significantly to the adherence of C. jejuni both, in vitro and in vivo.

One of the first C. jejuni proteins described to be involved in bacterial adhesion is Peb1A which represents the periplasmic binding protein component of an aspartate/glutamate ABC transporter. In addition this protein could be shown to mediate adherence to HeLa cells and to be important for the colonization of mice and, therefore, is believed to possess a dual function (Pei and Blaser, 1993; Pei et al., 1998; Leon-Kemps et al., 2006; Müller et al., 2007). However, the generation of a corresponding knockout mutant in the same C. jejuni strain (81-176) by another group revealed no differences in cellular adhesion. This discrepancy might be explained by the utilization of different cell lines or the conditions of their respective bacterial invasion assays (Novik et al., 2010). The C. jejuni N-glycosylated protein Peb3 has also been shown to serve as an adhesion protein but is also suggested to be a transport protein which might be involved in utilization of 3-phosphoglycerate (Min et al., 2009). Furthermore, JlpA, a N-linked glycosylated lipoprotein of C. jejuni which is exposed to the surface of the bacterium, mediates adhesion to Hep-2 cells via heat shock protein (Hsp) 90a. This particular interaction activates signalling pathways leading to NFkB and p38 MAP kinase activation which indicates an involvement in the inflammatory host cell response following C. jejuni infection. Indeed, the results of another group investigating the role of JlpA in cellular adherence of C. jejuni are not consistent. Knockout mutants of JlpA gave different results regarding their potential to adhere to host cells. The reasons for this might be the same as in the case of Peb1A mentioned above (Jin et al., 2001, 2003; Scott et al., 2009; Novik et al., 2010). CapA represents a surface-localized lipoprotein of C. jejuni, which mediates adherence to Caco-2 cells and plays an important role in the colonization of the chicken gut (Ashgar et al., 2007). Another protein, Cj0091 is important for the adherence of C. jejuni to INT407 cells (later identified as HeLa contaminated cell line) and also contributes to the colon-
nization of chicken (Oakland et al., 2011). In addition, Cj0268c enhances the adherence to Caco2 cells although this protein seems to be localized in the periplasmic space with no access of its C-terminus to the bacterial surface and a directed mutagenesis of gene cj0497 indicated this gene to encode for a potential adhesion protein (Javed et al., 2010; Tareen et al., 2013). However, for Cj0268c an involvement in C. jejuni induced immuno-
opathogenesis was demonstrated in vivo (Heimesaat et al., 2014). The flagellar apparatus of C. jejuni has been shown to secret proteins into the extracellular milieu. One of these proteins is FlaC, which is homologous to the N- and C-termini of flagellin A and B, respectively, but without the central part of these proteins. FlaC has been demonstrated to bind to Hep-2 cells and, for this, can be judged as an adhesion since FlaC mutants have a strongly reduced capacity to invade host cells (Song et al., 2004). Homologs of the CJIE1 prophage are detected in a significant percentage of C. jejuni isolates. While the patient symptoms were not affected by the presence or absence of this prophage, cell culture assays indicated an increased adherence in C. jejuni strains carrying CJIE1 (Clark et al., 2012). A functional type VI secretion (T6SS) system was recently identified in a chosen number of C. jejuni isolates. This system has been shown to play an important role for the adherence of the pathogen to human T84 colon epithelial cells as well as to murine RAW 264.7 macrophages since non-random knockout mutants of the T6SS genes hcp1 and comB3 resulted in a clearly adherence-reduced phenotype relative to the parental strain (Lertpiriyapong et al., 2012). A minority of C. jejuni strains carries a plasmid termed pVir. This plasmid encodes genes which are involved in DNA uptake or the transport of proteins by a putative type IV secretion system. Mutations of two of the plasmid encoded genes, comB3 and virB11, reduced the adherence to INT407 cells significantly (Bacon et al., 2000). Some adhesion proteins of C. jejuni have been shown to interact with the extracellular matrix component fibronectin which, on his part, binds to membrane spanning integrins. Major outer membrane protein MOMP of C. jejuni, for example, is able to bind to fibronectin, but also to the membrane of INT407 cells (Moser et al., 1997). Recently, it could be shown that MOMP is O-glycosylated, which significantly affects the conformation of the molecule. For this, O-glycosylation maintains the three-dimensional structure of MOMP which allows binding to histo-blood group antigens (BsAgs) but also promotes adhesion to host cells (Mahdavi et al., 2014). In addition, CadF, another outer membrane protein, has been shown not only to interact with fibronectin. It possesses bifunctional qualities as it facilitates a time-dependent activation of the small Rho family GTases Rac1 and Cdc42 (Konkel et al., 1997; Krause-Gruszczynska et al., 2007). Furthermore, also FliA has the property to mediate the attachment of the pathogen to epithelial cells by fibronectin binding and plays an important role for the colonization of chicken (Flanagan et al., 2009; Konkel et al., 2010). Thereby it is possible that the binding of the C. jejuni adhesins CadF and FliA to fibronectin promote the activation of the α5β1-integrin receptor as an initial step for bacterial invasion (Eucker and Konkel, 2012).

Adhesion proteins that directly interact with epithelial cells mediate host cell adherence by C. jejuni. To facilitate this particular function, the correct folding of these pro-

Capsule polysaccharides (cps) have obviously been shown to be essential for C. jejuni virulence including adherence, since a functional knockout of the kpsE gene encoding a transporter for cps almost eliminates the attachment of the mutant strain to INT-407 cells (Bacon et al., 2001; Bachtiar et al., 2007). Furthermore, C. jejuni cocultured with epithelial cells down-regulates the transcription of genes for the synthesis of cps significantly, which leads to a clearly diminished adherence to HCT-8 epithelial cells (Corcionivoschi et al., 2009). The adherence capacity of cps was also demonstrated as a non-capsulated mutant of C. jejuni strain 81-176 showed an almost 10-fold decrease in adherence compared to the wild-type strain (van Alphen et al., 2014).

**C. jejuni components for cellular invasion**

The invasion of intestinal cells by C. jejuni is believed to be the most important reason for colon damage and accompanying diarrheal disease. Infant monkeys infected with C. jejuni developed diarrhea whereby intracellular bacteria were detected in membrane bound vacuoles and free in the cytoplasm. In addition, damaged epithelial cells could be localized in the lumen of the colon (Russel et al., 1993). The outcome of experiments to investigate the C. jejuni invasion of host cells is not unique, but differs regarding the contribution of components that constitute the host cell cytoskeleton. C. jejuni invasion of INT407 cells could be blocked by the depolymerization of microtubules but not microfilaments. This observation was supported by another study, which detected a colocalization of C. jejuni not only with microtubules but also with motor protein dynein (Oelschlaeger et al., 1993; Hu and Kopecko, 1999). In contrast, during the invasion of Hep-2 cells, C. jejuni attachment could be seen in areas with an intracellular network of actin-like filaments indicating microfilament involvement. Furthermore, microfilament depolymerizing chemicals could significantly reduce invasion of INT407 cells (De Melo et al., 1989; Monteville et al., 2003). Taken together, the exact mode of cellular invasion by C. jejuni might depend and vary on the interplay between particular bacterial strains with their particular host cells.

Many proteins, but also carbohydrate components have been associated to the process of invasion. However, it cannot be excluded that in case of carbohydrate structures like sialylated lipooligosaccharides or capsular polysaccharides at the bacterial surface, their particular contribution to cellular invasion might be due to adherence rather than to entry of C. jejuni into the host cell (Bacon et al., 2001; Kanipes et al., 2004; Bachtiar et al., 2007; Louwen et al., 2008).

In contrast to other enteropathogenic bacteria like *Yersinia enterocolitica* or *E. coli*, C. jejuni is not equipped with a type-III-secretion system for direct injection of effector proteins into the host cell. Instead, C. jejuni secretes proteins via a type-III-homologue secretion system in its flagellar apparatus into the surrounding environment. These proteins are designated as *Campylobacter* invasion antigens (Cia) which are required for the invasion of and intracellular survival in host cells (Konkel et al., 1999). CiaB, localized in the cytoplasm of the host cell, was reported to be important for the process of...
invasion but not for adherence of \(C.\) \(jejuni\). Furthermore, \(\text{CiaB}\) seems to be involved in bacterial protein secretion itself since a \(\text{ciaB}\) knockout mutant has lost the ability to release eight proteins into the culture medium. Unfortunately, the invasion-relevant phenotype of \(\text{CiaB}\) could not be confirmed by Novik et al. (2010), where the \(\text{ciaB}\)-deficient mutant did not show altered invasion compared to the wildtype. The differences in the results of the respective groups might eventually be explained by the usage of different cell lines to determine bacterial invasion. Furthermore, since the invasion-deficient mutant with the strongly reduced invasion capacity was not functionally complemented, an alteration within the genome incoherent of the site directed mutation might be responsible for their lack of infectivity (Konkel et al., 1999; Novik et al., 2010). \(\text{CiaC}\) is another flagella-secreted protein which has been investigated regarding its function as a virulence factor of \(C.\) \(jejuni\). This protein is translocated into host cells following bacteria-host cell association. Thereby, similar to \(\text{CiaB}\), this protein is important for cellular invasion but not adherence. It exhibits a restricted secretion profile with the lack of one protein in a \(\text{ciaC}\) mutant. Furthermore \(\text{CiaC}\) is partially responsible for the recruitment of \(\text{Rac1}\) at cellular sites associated with \(C.\) \(jejuni\) which, in turn, mediates rearrangements of the host cell cytoskeleton and membrane ruffling (Christensen et al., 2009; Neal-McKinney and Konkel, 2012; Eucker and Konkel, 2012). Recently, \(\text{CiaD}\) has been investigated regarding its particular contribution on cellular invasion by \(C.\) \(jejuni\). Thereby, it was found that \(\text{CiaD}\) is involved in \(\text{Erk1/2}\) and \(p38\) activation which promotes the release of interleukin-8. Furthermore the \(\text{CiaD}\)-supported activation of MAP kinase \(\text{Erk1/2}\) facilitates the invasion by \(C.\) \(jejuni\) by phosphorylation of cortactin, known to promote polymerization and rearrangement of the cytoskeleton (Konkel et al., 2013; Samuelson and Konkel, 2013). Finally the protein \(\text{CiaI}\) was demonstrated to enable the intracellular survival of \(C.\) \(jejuni\) in so called \(\text{Campylobacter}\)-containing vacuoles (CCV; Buelow et al., 2011).

The results regarding the invasion capacity of protein \(\text{Cj0997}\) are incoherent. In a first publication, a \(\text{cjo997}\) mutant was described to be invasion deficient, but to be fully motile. A later study confirmed the strongly reduced capacity of the mutant to invade host cells, but, in contrast to the first publication, the mutant exhibited reduced motility. This would indicate that the reduced invasiveness is due to its diminished motility, which might be correlated with the absence of this protein in an unknown fashion (Goen et al., 2006; Novik et al., 2010). Like gene \(\text{cjo997}\), several other genes are under \(\text{a28}\) control, which governs the transcription of \(\text{flaA}\) but also other genes for the constitution of the flagellar apparatus. These genes have been designated as flagellar coexpressed determinants (feds). Investigations of the corresponding mutants detected a colonization-reduced phenotype in mice for several fed genes, but only \(\text{feda}\) was shown to be important for the invasion of host cells (Barbero-Tobon and Hendrixson, 2012). The cytotoxic lethal distending toxin (CDT) has been shown to mediate cell cycle arrest (Lara-Tejero and Galan, 2000). The role of \(C.\) \(jejuni\) CDT for the process of invasion seems to depend on the origin of the respective host cell line. While CDT mutants were clearly reduced in their potential to invade human HeLa cells, no difference between wildtype strain and mutant was observed applying avian macrophage HD-11 cells (Biswa et al., 2006; Jain et al., 2008). Another \(C.\) \(jejuni\) protein that was shown to be important for cellular invasion is \(\text{Cj1496c}\). Comparison of an insertion mutant as well as an in-frame deletion mutant with the wildtype strain, revealed both mutants to be defective for the invasion of INT-407 cells (Kakuda and DiRita, 2006). The recently described type VI secretion system was also shown to be relevant for invasion. However as mentioned above, mutations of the \(\text{T6SS}\) exhibited an adherence-reduced phenotype which might be the actual reason for reduced invasion. This might also be the case regarding the invasion capacity of prophage homolog \(\text{CjIE1}\) (Clark et al., 2012; Lertpiriyapong et al, 2012).

Due to the detection and characterization of all these components that participate in the invasion of host cells by \(C.\) \(jejuni\), our knowledge how this important pathogen facilitates cellular uptake has grown. However, further genes have been detected that contribute to the invasion process (Novik et al., 2010). But even these virulence factors together with the ones already characterized may not represent the complete picture yet.

**Conflict of interest**

The authors declare that no competing interests exist.

**References**


Tareen AM, Dasti JI, Zautner AE, Groß U, Lugert R (2010): Campylobacter jejuni proteins Cj0268c and Cj0951c affect the chemotaxic behavior towards formic acid and are important for the invasion of host cells. Microbiology 156: 3123–3135.


Address for correspondence:
Dr. Raimond Lugert
University Medical Center Göttingen
Institute for Medical Microbiology
Kreuzbergring 57
37075 Göttingen
Germany
rlugert@gwdg.de