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Review

Effects of antibiotic resistance (AR) and microbiota shifts on *Campylobacter jejuni*-mediated diseases

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Abstract

Campylobacter jejuni is an important zoonotic pathogen recently designated a serious antimicrobial resistant (AR) threat. While most patients with *C. jejuni* experience hemorrhagic colitis, serious autoimmune conditions can follow including inflammatory bowel disease (IBD) and the acute neuropathy Guillain Barré Syndrome (GBS). This review examines inter-relationships among factors mediating *C. jejuni* diarrheal versus autoimmune disease especially AR *C. jejuni* and microbiome shifts. Because both susceptible and AR *C. jejuni* are acquired from animals or their products, we consider their role in harboring strains. Inter-relationships among factors mediating *C. jejuni* colonization, diarrheal and autoimmune disease include *C. jejuni* virulence factors and AR, the enteric microbiome, and host responses. Because AR *C. jejuni* have been suggested to affect the severity of disease, length of infections and propensity to develop GBS, it is important to understand how these interactions occur when strains are under selection by antimicrobials. More work is needed to elucidate host–pathogen interactions of AR *C. jejuni* compared with susceptible strains and how AR *C. jejuni* are maintained and evolve in animal reservoirs and the extent of transmission to humans. These knowledge gaps impair the development of effective strategies to prevent the emergence of AR *C. jejuni* in reservoir species and human populations.

Keywords: *Campylobacter jejuni*, Guillain-Barré Syndrome, microbiome, mouse models, autoimmunity, inflammation, antibiotics, antimicrobial resistance.

Introduction

Campylobacter jejuni continues to cause disease worldwide despite several decades of efforts to control these infections (CDC, 2017). The concerning rise of AR *C. jejuni* incidence predicts future increases in enteritis cases and enhanced risk of Guillain Barré Syndrome (GBS) (CDC, 2013). Because *C. jejuni* is a broad host range pathogen that infects animals and subsequently humans, a major gap in our understanding is curbing infection in animals (Oliver *et al.*, 2009). Despite many efforts, simple hygiene or biological control measures in food animal production environments have failed to deliver adequate control of *C. jejuni* carriage in animals (Oliver *et al.*, 2009; Newell *et al.*, 2011). A better understanding of the interactive factors mediating *C. jejuni* colonization and disease in animals and people is needed.

Interactive factors mediating enteric disease were poorly understood until the age of high throughput analysis of bacterial transcriptomics, host responses, and microbial communities. These tools have spurred studies to examine these factors together for their roles in *Campylobacter* disease pathogenesis. For example, mechanistic work in human and mouse models has informed inter-relationships among factors promoting

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colonization or enhancing disease, including *C. jejuni* virulence factors and antimicrobial resistance, the enteric microbiome, and host responses controlling susceptibility, resistance, or autoimmunity. It is now well known that significant genetic variation occurs in virulence and lipooligosaccharide (LOS) loci between *C. jejuni* strains from enteritis and GBS patients (Godschalk *et al.*, 2007) and that these modifications mediate a switch in host adaptive responses and disease phenotype (Malik *et al.*, 2014). For example, the GBS strain 260.94 can elicit type 2 responses with IgG1 autoantibodies against GM1 and GD1a nerve gangliosides due to sialylation of the outer core of the LOS mimicking these structures, while most isolates from patients with enteritis do not have these modifications and cannot provoke these responses (Malik *et al.*, 2014). Yet the exact mechanisms controlling these different syndromes are largely unknown.

Likewise, little is known about AR *C. jejuni*. Few studies focus on how they are maintained in and evolve in animal reservoirs and the extent to which they are transmitted to humans. Furthermore, little experimental work has been done to elucidate the host–pathogen interactions of AR *C. jejuni* and how they compare with susceptible strains. In fact, previous opinions upheld the concept that antibiotic resistance carriage was associated with a fitness cost for *C. jejuni* (Almofti *et al.*, 2011), but this varies according to the resistance-conferring mutation (Zhang *et al.*, 2006). Many studies now indicate that antibiotic resistance is able to enhance *C. jejuni* fitness *in vivo* (Zhang *et al.*, 2003; Luo *et al.*, 2005) yet the effect of this on virulence during *in vivo* infections has only been lightly explored (Moore *et al.*, 2006). Recent work linking AR and other factors to *C. jejuni* disease have been conducted in animal models. Animals used to study the pathogenesis of *C. jejuni* infection include mice, rats, rabbits, pigs, chickens, and ferrets (Newell, 2001; Mansfield *et al.*, 2007; 2008b). Mice provide many advantages including (1) low cost to maintain, (2) small space requirements for housing, allowing larger samples sizes, (3) ease of manipulation, and (4) availability of genetic knockouts. Development of murine *C. jejuni* colonization and colitis models has been greatly advanced by manipulation of host genetics and host microbiota (Chang and Miller, 2006; Mansfield *et al.*, 2008a; Bereswill *et al.*, 2011; Malik *et al.*, 2014; Stahl *et al.*, 2014; O'Loughlin *et al.*, 2015; Brooks *et al.*, 2017). A summary of these advances is also provided, highlighting interactive factors mediating disease after *C. jejuni* oral infection (Fig. 1). Such comparative analysis of *C. jejuni* disease in humans and mouse models can aid in understanding these complex relationships, especially the effects of AR on virulence.

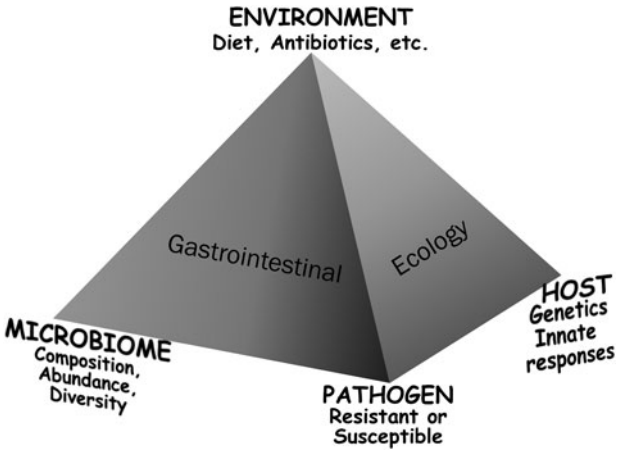

Figure 1. The major factors that interact to produce gastrointestinal disease. Host innate responses coupled with the gut microbiota can produce colonization resistance to certain enteric pathogens. Diet and other environmental factors such as antibiotic treatment can modify the microbiome and select for pathogen resistance. Many other interactions are possible that have yet to be explored experimentally.

Epidemiology of susceptible and AR *C. jejuni*: human and animal studies

Human studies

C. jejuni is an important zoonotic pathogen causing 1.3 million cases of hemorrhagic gastroenteritis annually in the USA, leading to 13,000 hospitalizations, 120 deaths (Scallan *et al.*, 2011) and many unreported sporadic cases. *Campylobacter* cases represented 9% of the 9.4 million episodes of foodborne illness reported in 2011, but it represented 15% of those cases requiring hospitalization. Human infection occurs via the oral route and most often results from the consumption of raw or undercooked poultry (Young *et al.*, 2007). Contaminated meat products and water, contact with pets, and international travel result in sporadic infections (Ketley, 1997; Altekruse *et al.*, 1999; Bopp *et al.*, 2003); outbreaks have been associated with unpasteurized milk and contaminated water (Ketley, 1997; Allos, 2001; Cawthraw *et al.*, 2002; Bopp *et al.*, 2003).

C. jejuni was recently designated a 'serious level' antimicrobial resistant threat by the Centers for Disease Control and Prevention (CDC) (CDC, 2013). Resistance to ciprofloxacin, a fluoroquinolone used to treat more severe infections, has increased in the USA from 13% in 1997 to 25% in 2011 (CDC, 2013) and was estimated to cause 310,000 of the 1.3 million infections each year (Scallan *et al.*, 2011). Moreover, in 2014 ciprofloxacin resistance was detected in 26.7% of *C. jejuni* human isolates, in 28% of *C. jejuni* chicken isolates and in more than 35% of *Campylobacter coli* isolates from human beings (FDA, 2014). Resistance to azithromycin, a commonly used macrolide, was also estimated to occur in 2% ($n = 22{,}000$) of *Campylobacter* infections in 2011 (Scallan *et al.*, 2011). Based on NARMS 2015 data the percentages of human *C. jejuni* isolates carrying resistance to other antibiotics included 46% tetracycline, 23% nalidixic acid, 9.9% florfenicol, 7.2% clindamycin, 1.9% telithromycin, 1.7% erythromycin, 0.9% gentamicin, and 0.8% chloramphenicol (CDC, 2017).

The use of antibiotics is the most important factor driving selection for antibiotic resistance with dominant applications occurring in healthcare, agriculture and the environment (Holmes *et al.*, 2016). Yet there is ongoing controversy regarding which practices in human medicine and veterinary medicine are

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most harmful in enhancing AR. In human medicine upwards of half of the prescribed antibiotics are unnecessary or misprescribed, while in food animal medicine the FDA has recommended phase-out of antibiotics for promoting growth (CDC, 2013). Notably, in most studies on antibiotic use, the risks to human health or the benefits to animal production have not been well studied (Landers *et al.*, 2012).

Animal studies

The gastrointestinal (GI) tract of domestic and wild animals is the natural ecological niche for *C. jejuni*, making it a broad host-range pathogen. Naturally occurring infections with *C. jejuni* have been reported in juvenile Rhesus monkeys (Fitzgeorge *et al.*, 1981), macaques (Sestak *et al.*, 2003), ferrets (Fox, 1992), dogs (Bruce *et al.*, 1980; Fox *et al.*, 1983) swine (Mansfield and Urban, 1996), and wild birds (Kaakoush *et al.*, 2015). Most avian species serve as asymptomatic reservoirs for *Campylobacter* and infect other birds through common water and feeding sources (Kaakoush *et al.*, 2015). Domestic poultry is colonized with *C. jejuni* without disease and are considered a principal risk factor for human infection (McCrackin *et al.*, 2016). This is not surprising because broiler flocks have a high rate of *C. jejuni* carriage in their gut microbiome resulting in a high level in retail poultry (Kaakoush *et al.*, 2015). In the USA, 50% of raw chicken in stores was contaminated with *Campylobacter*, likely due to transfer during slaughter and processing. Since 2007, 35% of *Campylobacter* outbreaks were caused by isolates from poultry, which was more than any other source (Kaakoush *et al.*, 2015). In a recent 2015 study, the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) estimated that transmission of 50–80% of all human cases of campylobacteriosis are related to chickens. Other food animals, such as cattle, sheep, and swine, also harbor *C. jejuni*. Thus, animal exposures and consumption of raw or unpasteurized milk and untreated water also contribute to *Campylobacter* infections (Young *et al.*, 2007).

Animals constitute one of the main reservoirs of AR *C. jejuni*. With increasing use of quinolone antibiotics as growth promoters in the poultry industry, isolation of fluoroquinolone-resistant strains has increased significantly since 1992 (Kaakoush *et al.*, 2015). While public health authorities of several countries, including the US FDA, have banned the use of fluoroquinolones for growth-promotion, such bans are not universal, and these drugs are still approved for treating infections in poultry. Unfortunately, the CDC reported in 2012 that the percentage of ciprofloxacin-resistant *Campylobacter* isolates from retail chicken has remained unchanged since the ban took effect. *Campylobacter* isolates from dairy cattle on farms managed organically and conventionally had similar patterns of antimicrobial resistance, but the proportion of resistant isolates was higher for conventional than organic farms (Halbert *et al.*, 2006). Resistance to seven of eight drugs (azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline) was very low and did not differ by farm type, but tetracycline resistance was 48% on organic and 58% on conventional dairy farms. Ten years later high rates of AR *C. jejuni* were isolated from three cattle herds in the same area of the Northeastern USA; 65.9% were resistant to tetracycline, 1.5% were resistant to macrolides, and 16.3% were resistant to ciprofloxacin (Cha *et al.*, 2017). Here, polymerase chain reaction (PCR)-based fingerprinting showed identical patterns between multilocus sequence type (ST)-982 isolates from cattle and people suggesting transmission. A large study examined the prevalence and antimicrobial resistance profile of *Campylobacter* spp. in conventional and antimicrobial-free swine production systems in the US. Investigators found that 472/838 (56.3%) of pigs were positive for *Campylobacter* spp., which did not vary based on the production system (conventional 58.9% and antimicrobial-free 53.7%) (Tadesse *et al.*, 2011). Antibiotic resistance was detected to tetracycline (64.5%), erythromycin (47.9%), and nalidixic acid (23.5%), but only erythromycin resistance was found to be higher on conventional than antibiotic-free farms. Furthermore, a highly virulent tetracycline-resistant *C. jejuni*, termed clone SA, has been identified and linked to abortion in sheep and cattle (Sahin *et al.*, 2008); this strain was also isolated from campylobacteriosis patients linked to raw cow's milk (Sahin *et al.*, 2012). Finally, in a northern Canada study, 58% of healthy dogs and 97% of diarrheic dogs were shedding *C. jejuni* in feces (Chaban *et al.*, 2010), but AR was not examined. In a similar Swiss study of healthy dogs, 6.3% had *C. jejuni*, 5.9% had *Campylobacter upsaliensis* and 0.7% had *Campylobacter coli*; no macrolide resistance was found, but 28 isolates (20.9%) were resistant to quinolones (Amar *et al.*, 2014). Here, 94% of the canine isolates had an ST that was also found in human clinical isolates. While these few examples suggest animal to human transmission, in-depth transmission studies have not been done. In a review of recent literature, it was discerned that on-farm antibiotic selection pressure does increase colonization of animals with drug-resistant *C. jejuni*, yet this has not yet been causally linked to the prevalence of drug-resistant foodborne enteric campylobacteriosis in human beings (McCrackin *et al.*, 2016). However, studies estimating the impact of therapeutic treatment with fluoroquinolones for respiratory diseases in cattle on antibiotic resistance in *Campylobacters* suggest the impact is exceedingly small (Hurd *et al.*, 2010). Considering data published on the animal to human transmission of *Campylobacter*, there is little consideration given to the role of animals in the transfer of strains that cause autoimmune diseases.

Campylobacter jejuni colonization of the host

Colonization of animals and human beings

Chickens are natural hosts for *C. jejuni*, and infection results in high-level asymptomatic GI colonization without inflammatory responses or signs of disease (Kaakoush *et al.*, 2015). (Hermans *et al.*, 2012). Transmission in chickens occurs very early in life and is extremely rapid, which may be due to palatine colonization leading to transmission through communal water troughs and standard fecal-oral spread (Montrose *et al.*, 1985;

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Pearson *et al.*, 1993). They mount detectable immune responses to *C. jejuni* that fail to limit colonization, and that is considered by some as a form of immunological tolerance to *C. jejuni*. Additionally, *Campylobacter* spp. have been isolated from free-living birds, including migratory birds and waterfowl, crows, gulls, and domestic pigeons (Hill and Grimes, 1984; Maruyama *et al.*, 1990). Despite this high rate of colonization, disease in naturally infected birds due to *C. jejuni* is rare but can occur especially in psitticine birds (Young and Mansfield, 2005). Weis *et al.* (2016) examined the genomes of *Campylobacter* spp. isolated from a broad range of animal hosts and showed that strains from human beings, non-human primates, chickens, cows, crows, goats, and sheep had some similarities. From their data, they postulated that 17% of *Campylobacter* spp. isolated from crows were highly similar to those isolated from human and nonhuman primates. These data show that environmental to human transmission is possible and provoke the need for an understanding of the eco-epidemiology of animal colonization with *Campylobacter* strains from different environments. Thus, more work is needed to understand host colonization mechanisms and zoonotic spread of this pathogen (Weis *et al.*, 2016). Furthermore, for control measures to be successful, it will be necessary to understand the mechanisms underlying colonization resistance whereby the intestinal microbiota protects itself against incursion by *C. jejuni*.

In many mammals, including human beings, *C. jejuni* colonizes the GI tract and initiates GI inflammation. After oral infections, *C. jejuni* surviving the acid environment of the stomach adhere to intestinal epithelial cells or to the mucus overlying these cells and replicate, which can result either in asymptomatic colonization status or diarrheal illness (Janssen *et al.*, 2008). Experimental evidence shows that *C. jejuni* gastroenteritis is associated with specific strains of *C. jejuni* (Bell *et al.*, 2009; Malik *et al.*, 2014) and can be enhanced by serial passage (Bell *et al.*, 2009), by depleting the microbiota with antibiotics (O'Loughlin *et al.*, 2015), or by infecting gnotobiotic animals (Chang and Miller, 2006). Recent studies have also shown that *C. jejuni*-mediated-autoimmunity is also enhanced under the environmental conditions employed in these models especially by depleting gut microbial communities with antibiotics (Brooks *et al.*, unpublished; (St Charles *et al.*, 2017).

The ability of commensal microbiota to prevent colonization by exogenous pathogens or opportunistic commensals has been termed "colonization resistance." Considerable recent work has been aimed at enhancing colonization resistance using anti-*Campylobacter* compounds including, probiotics, bacteriophages, vaccines, and anti-*Campylobacter* bacteriocins, all of which may be successful at reducing cfu loads in poultry (Johnson *et al.*, 2017). Phenolic compounds have also been tested producing significant but variable activities against AR and susceptible *C. jejuni* strains (Klancnik *et al.*, 2012). Interestingly, some phenolic compounds significantly reduced the expression of the CmeABC efflux pump imparting enhanced sensitivity to antibiotics (Oh and Jeon, 2015). Many other natural compounds from plants and other organisms are under study for inhibiting *C. jejuni* colonization. More basic work is needed to understand interactions of specific treatments with *C. jejuni* AR response elements.

Colonization rates for AR and susceptible C. jejuni

Several studies have been conducted to examine the colonization rates of AR versus susceptible *C. jejuni*. Luo and colleagues examined the effect of fluoroquinolone resistance on colonization and fitness of *C. jejuni* in the chicken host (Luo *et al.*, 2005). They found that both resistance and susceptible *C. jejuni* had similar colonization levels and persistence in specific pathogen-free White Leghorn chickens when no fluoroquinolone antibiotics were given. However, upon co-inoculation of resistant and susceptible strains in the presence of fluoroquinolone treatment, the resistant isolates outcompeted the majority of the FQ-susceptible strains, showing enhanced fitness. This fitness advantage was the single point mutation in *gyrA* and was not due to compensatory mutations in the genes targeted by FQ. In a similar competition trial, Luangtongkum *et al.* (2012) tested erythromycin resistant and susceptible strains for their ability to colonize and persist in newly hatched broiler chickens. They found that erythromycin susceptible *C. jejuni* strains repeatedly outcompeted the resistant strains when antibiotics were not used, suggesting that macrolide-resistant strains will likely decrease in the absence of antibiotic selection pressure. Clearly, more information is needed to understand colonization resistance mechanisms in relation to AR *C. jejuni* strains.

Persistent C. jejuni *colonization results in inflammation*

Studies to understand colonization factors for *C. jejuni* have been done mainly in chickens because of its importance as a reservoir for human infection, yet chickens are not known to progress from colonization to disease as occurs in human beings. Thus, mouse models have been employed to study inflammation after *C. jejuni* colonization. Limited enteric flora (LF) C3H severe combined immune deficient (SCID) mice infected with *C. jejuni* displayed high-level *C. jejuni* colonization for up 224 days. In contrast, immune competent congenic LF C3H mice began to clear the bacteria at approximately 28 days (Chang and Miller, 2006). LF C3H SCID mice, but not LF C3H immune competent mice, displayed inflammation of the cecum and the colon (Chang and Miller, 2006), suggesting that inflammation may allow *C. jejuni* to persist in the gut. This explanation would be consistent with experimental data from other pathogens including *Salmonella enterica serovar Typhimurium* (Winter *et al.*, 2010) and some pathogenic *Escherichia coli* (Horwitz and Silverstein, 1980) that have evolved mechanisms to exploit inflammation by utilizing tetrathionate and evading complement fixation, respectively. In general, two hypotheses exist to explain how enteric pathogens may benefit from inflammation: (1) inflammation alters microbiota structure in a way that frees up nutrients that are exploited by pathogens but not the microbiota (i.e. food hypothesis) and (2) changes in antimicrobial compounds produced by the inflamed tissue may be detrimental to the microbiota but not the pathogen (i.e. differential killing hypothesis) (Stecher and Hardt, 2008).

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Dysbiosis and susceptibility to pathogens such as C. jejuni

Dysbiosis is a term used to describe microbial communities that are depleted of beneficial bacteria; such depletion is associated with increased susceptibility to both pathogen-mediated and non-pathogen associated diseases. Dysbiosis may result from immune deficiencies, changes in diet, antibiotic treatment, and acute inflammation (Honda and Littman, 2012). One consequence of dysbiosis in people and animals includes diminished pathogen colonization resistance (Buffie and Pamer, 2013). When compared with healthy individuals, dysbiosis has been found in patients with various chronic inflammatory and autoimmune diseases, including inflammatory bowel disease (IBD), multiple sclerosis, and Type 1 diabetes (Ercolini and Miller, 2009). In some cases, these diseases have direct links to pathogenic organisms; however, others are associated with fluctuations in the abundance of particular commensal microorganisms (Shimon, 2000; Ercolini and Miller, 2009; Chervonsky, 2013). Obligate anaerobes likely play a critical role in colonization resistance to pathogens. Depletion of these organisms may free up nutrients for fast growing organisms including *Proteobacteria* (van der Waaij *et al.*, 1971, 1972; Wells *et al.*, 1988; Shin *et al.*, 2002). To evaluate the role of the microbiota in pathogen-associated diseases, germfree, gnotobiotic, and antibiotic-depleted microbiota mice have been experimentally infected and some are described in the following sections. It is well established that dysbiosis can enhance susceptibility to pathogens, increasing the likelihood of disease in the host following infection with *S. Typhimurium, E. coli,* and *C. jejuni.*

Microbiota mediated colonization resistance

Early attempts to develop a murine model of *C. jejuni* colonization and colitis were retarded by an inability to colonize wild-type (WT) mice with *C. jejuni* or low-level *C. jejuni* colonization with subclinical disease (Chang and Miller, 2006; O'Loughlin *et al.*, 2015). *C. jejuni* colonization resistance was abolished by infecting gnotobiotic mice; and persistent, high-level *C. jejuni* colonization was achieved by infecting immune deficient gnotobiotic mice (Chang and Miller, 2006). Along with persistent *C. jejuni* colonization, immune deficient (i.e. SCID) gnotobiotic mice had marked inflammation of the cecum, colon, and stomach (Chang and Miller, 2006). Other reports showed that both specific pathogen-free (SPF) C57BL/6 wild-type and congenic C57BL/6 interleukin-10 deficient (IL-$10^{-/-}$) mice were colonized by *C. jejuni,* but only IL-$10^{-/-}$ mice were susceptible to colitis after infection with *C. jejuni* 11168 (Mansfield *et al.*, 2007, 2008a). In this work, it was also shown that presence of *Helicobacter hepaticus* or related mouse pathogens conferred immunological resistance to colonization with *C. jejuni* strains. The C57BL/6 IL-$10^{-/-}$ mouse model has also been used to determine if the microbiota plays a critical role in the inflammation seen in *C. jejuni*-infected C57BL/6 IL-$10^{-/-}$ mice.

Shifts in microbiota enhance colonization of *C. jejuni*

Inoculation of mice with human fecal material has been used to generate humanized microbiota mice (^{Hu}microbiota). In one model, ^{Hu}microbiota mice were generated by using a five-antibiotic cocktail (ampicillin, vancomycin, ciprofloxacin, imipenem, and metronidazole) to deplete the microbiota, followed by inoculation with either murine or human feces. Per oral *C. jejuni* infection of these mice resulted in clearance of *C. jejuni* in 2 days by murine microbiota mice. In contrast, mice with human microbiota remained colonized for 6 weeks and displayed exacerbated T cell, B cell, and pro-inflammatory cytokine responses in the colonic mucosa (Bereswill *et al.*, 2011). However, murine microbiota controls were also pretreated with antibiotics thus raising the question of whether this affected their immune responses.

Transplanted human fecal microbiota from young adults (^{Hu}microbiota) altered immune responses to *C. jejuni* infection in C57BL/6 mice when compared with congenic mice with conventional mouse microbiota (^{Conv}microbiota) (Brooks *et al.*, 2017). ^{Hu}microbiota and ^{Conv}microbiota mice had statistically significant differences between their microbial communities, although alpha diversity indices showed no differences in diversity between experimental groups. C57BL/6 mice carrying a stable ^{Hu}microbiota and experimentally infected with *C. jejuni* strains from a colitis patient and a GBS patient had higher *C. jejuni* colonization levels and colonic inflammation scores than congenic mice with ^{Conv}microbiota. *C. jejuni* 11168, but not 260.94, elicited T_H1- and T_H17-associated anti-*C. jejuni* antibody responses. Notably, ^{Hu}microbiota mice displayed a T_H2 biased anti-*C. jejuni* antibody response independent of inoculation status. Finally, ^{Hu}microbiota mice infected with both *C. jejuni* patient strains had elevated GM1 anti-ganglioside antibody responses, but only those given strain 260.94 were significantly higher than conventional microbiota mice given the same strain. These data demonstrate that human microbiota alters host–pathogen interactions in infected mice increasing colonization and autoimmune responses in a *C. jejuni* strain-dependent manner. Thus, particular microbiota compositions are likely to enhance host susceptibility to GBS following *C. jejuni* infection.

Disease associated with *C. jejuni*: hemorrhagic gastroenteritis and IBD in human beings

C. jejuni causes a spectrum of diseases in people with some individuals colonized but asymptomatic. However, most patients ingesting *C. jejuni* in undercooked meat or unpasteurized milk develop mild-to-severe gastroenteritis targeting the colon, which is debilitating but self-limiting within 7–10 days (Vandenberg *et al.*, 2013). Lesions include colonic crypt distortion, crypt abscesses, mucin depletion, edema of the colonic lamina propria, and significant infiltration of granulocytes and mononuclear cells (Young and Mansfield, 2005). Infection initiated in the GI tract can become extra-intestinal, particularly in immune-compromised hosts (Karmali and Fleming, 1979;

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Blaser *et al.*, 1980; Ketley *et al.*, 1996). Damage resolves in most patients, but campylobacteriosis can be life-threatening in immune-compromised or HIV-infected people with persistence, systemic spread, and multi-organ damage (Young and Mansfield, 2005; Fernandez-Cruz *et al.*, 2010). Based on CDC data, these infections are more likely to lead to death (CDC, 2011). AR *C. jejuni* have been suggested to cause more severe infections requiring lengthier hospitalizations when compared with susceptible infections (Moore *et al.*, 2006) and, thus, represent an important public health concern. Macrolides and fluoroquinolones are the antibiotics of choice for treating *C. jejuni* infections, while tetracyclines are sometimes considered as alternatives to these drugs but are rarely used. In cases of bacteremia or multi-organ infection, intravenous amino glycosides are often used. Based on the rising incidence of resistance to these drugs, Danish scientists compared adverse health events associated with susceptible and AR *C. jejuni* infection in 3471 patients (Moore *et al.*, 2006). Patients with quinolone-resistant strains had a 9.68-fold increased risk of adverse events within 30 days, while patients with erythromycin-resistant strains had a 5.51-fold risk of an adverse event within 90 days when compared with patients with susceptible strains of *C. jejuni*. More work is needed to understand whether this enhanced disease is due to AR alone or whether virulence attributes of these strains are enhanced in some other manner such as selection compounded by dysbiosis. Given the rising incidence of AR *C. jejuni* in people and animals, this is a key question to address keeping in mind that multiple interactions may be at work.

The inflammatory nature of *Campylobacter* enteritis, along with compatible endoscopic or histopathologic findings, can produce a clinical picture that resembles IBD (Farmer, 1990; Perkins and Newstead, 1994). Fecal erythrocytes and leukocytes are present in the majority of campylobacteriosis cases whether or not the stools are grossly bloody. In several studies, investigators have shown that acute *C. jejuni* gastroenteritis is followed by an increased risk for IBD (Garcia Rodriguez *et al.*, 2006) (Ternhag *et al.*, 2008). Other closely related strains such as *Campylobacter concisus* have also been linked to the development of IBD (Hold *et al.*, 2014).

T helper-1 based inflammation is associated with *C. jejuni*-induced hemorrhagic colitis

IL-10 is classified as an anti-inflammatory cytokine that down-regulates host response to invasion by intracellular pathogens by inhibiting several key inflammatory regulators, including major histocompatibility complex II and T-cell co-stimulatory factors B7-1 and B7-2, and expression of interferon (IFNγ) (Moore *et al.*, 2001; Ouyang *et al.*, 2011). Congenic C57BL/6 IL-10 deficient mice (C57BL/6 IL-$10^{-/-}$) but not their IL-$10^{+/+}$ counterparts were susceptible to colitis when infected by *C. jejuni* 11168 (Mansfield *et al.*, 2007). *C. jejuni* 11168 successfully colonized the GI tract of C57BL/6 WT and IL-$10^{-/-}$ mice; however, only IL-$10^{-/-}$ mice developed inflammation of the colon and cecum. The cecum was the GI site with the highest level of colonization; and *C. jejuni* was isolated from most GI compartments (i.e. cecum, stomach, colon, and jejunum) or detected by *C. jejuni* specific (*gyr*A) PCR of tissue homogenates. All mice were colonized at comparable levels and colonization was necessary but not sufficient for GI lesions as only IL-$10^{-/-}$ mice developed disease and lesions (Mansfield *et al.*, 2007).

Colitis in IL-$10^{-/-}$ mice was *C. jejuni* strain dependent (Bell *et al.*, 2013a; Malik *et al.*, 2014), and genomic composition of the *C. jejuni* strain was an important factor in determining the disease outcome (Bell *et al.*, 2013a). To date the entire suite of genes required for *C. jejuni* colitis remains unknown; however, comparative genomics of available *C. jejuni* genomes and gene expression analysis of *C. jejuni* strains that caused colitis in C57BL/6 IL-$10^{-/-}$ mice compared with those that did not yield 201 potential virulence genes, collectively called the *C. jejuni* virulome (Bell *et al.*, 2013a). Motility is a major determinant of *C. jejuni* pathogenesis. *C. jejuni* diminished motility and non-motile mutants colonized at rates 100 to 1000-fold less than the WT (Wassenaar *et al.*, 1993) thus variation in motility amongst strains played a role in infection outcomes. Further, an experiment in *C. jejuni* 11168-infected germ-free C57BL/6 mice showed that expression levels of 90 open reading frames (ORFs) were significantly up- or down-regulated in the mouse cecum at least 2-fold compared with *in vitro* growth (Bell *et al.*, 2013b). Genomic content of these ninety *C. jejuni* 11168 ORFs was significantly correlated with the capacity to colonize and cause enteritis in mice. Differences in gene expression levels and patterns are thus an important determinant of pathotype in *C. jejuni* strains in this mouse model and more work is needed to reveal the function of many of these virulence factors.

Mouse *C. jejuni* disease models have demonstrated that most strains are invasive and elicit strong inflammatory responses particularly in the colon (Chang and Miller, 2006; Mansfield *et al.*, 2007; 2008a). Many *C. jejuni* strains causing disease in these models have cytolethal distending toxin and produce effacing lesions that would be expected to release many self-antigens (Pickett *et al.*, 1996; Mansfield *et al.*, 2007, 2008a). Upon invasion, most *C. jejuni* strains are captured and processed by DCs in the lamina propria (Rathinam *et al.*, 2008, 2009). BM-DCs challenged with *C. jejuni* efficiently internalized and killed *C. jejuni* 11168 and significantly upregulated surface MHC-II, CD40, CD80 and CD86 demonstrating a mature phenotype. Infected BM-DCs secreted significant amounts of TNFα, IL-6 and IL-12p70. Maximal activation of murine BM-DCs required internalization of *C. jejuni*; attachment alone was not sufficient to elicit significant responses. Also, various strains of *C. jejuni* elicited different magnitudes of cytokine production from BM-DCs. TLR2, TLR4, MyD88, and TRIF also played a role in *C. jejuni*-induced inflammatory activation of murine DCs (Rathinam *et al.*, 2009). DC upregulation of MHC-II and costimulatory molecules after *C. jejuni* challenge was profoundly impaired by TLR2, TLR4, MyD88, and TRIF deficiencies. Similarly, *C. jejuni*-induced secretion of IL-12, IL-6, and TNFα was significantly inhibited in TLR2$^{-/-}$, TLR4$^{-/-}$, MyD88$^{-/-}$, and TRIF$^{-/-}$ DCs compared with WT DCs. Furthermore, *C. jejuni* infection induced IRF-3 phosphorylation and IFN-β

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secretion by DCs in a TLR4-TRIF dependent fashion, further demonstrating activation of this pathway by *C. jejuni*. Importantly, TLR4, MyD88, and TRIF deficiencies markedly impaired Th1-priming ability of *C. jejuni*-infected DCs. These results showed that *C. jejuni*-induced signaling through TLR4-MyD88 and TLR4-TRIF axes mediated maturation and inflammatory responses of DCs. Finally, in a coculture system, *C. jejuni* -infected BM-DCs induced high-level IFNγ production from CD4 + T cells indicating Th1 polarization. This finding correlates with *in vivo* studies demonstrating Th1-associated IgG2b antibody responses in IL-$10^{+/+}$ and IL-$10^{-/-}$ mice of C57BL/6 (Mansfield *et al.*, 2007), C3H and non-obese diabetic (NOD) genetic backgrounds (Mansfield *et al.*, 2008a) and in C57BL/129 mice (Fox *et al.*, 2004) challenged orally with *C. jejuni*. However, excessive innate or T-cell mediated inflammatory responses in the intestine triggered by DCs in the absence of immunoregulatory elements, like IL-10, contribute to immune pathology as evident in the C57BL/6 IL-$10^{-/-}$ enteritis model with lesions indistinguishable from human Crohn's disease patients (Mansfield *et al.*, 2007, 2008a; Bell *et al.*, 2009).

Humphrey *et al.*, infected four commercial breeds of broiler chickens with *C. jejuni* M1 strain and demonstrated that some chickens experience disease and inflammation (Humphrey *et al.*, 2014). Infected birds of all four breeds mounted an innate immune response similar to that seen in mammals, which was controlled in most breeds by upregulation of IL-10. Prolonged inflammatory responses were seen in one chicken breed along with diarrhea and GI lesions. Despite this large body of experimental infection studies in animals including chickens, little is known about whether AR *C. jejuni* strains elicit enhanced inflammatory responses post infection.

Disease associated with *C. jejuni*: GBS and autoimmunity in humans

C. jejuni has also been linked to the peripheral neuropathies, GBS and Miller Fisher Syndrome, as well as IBD, irritable bowel syndrome (IBS), and reactive arthritis (RA). All are significant autoimmune conditions associated with recent *Campylobacter* infection with GBS considered the leading cause of acute neuromuscular paralysis worldwide (Willison and Plomp, 2008). A recent meta-analysis by Keithlin *et al.* (Keithlin *et al.*, 2014) estimated the incidence of GBS following *C. jejuni* infection to be 7 per 10,000, while rates of IBS and RA were higher at 40 per 1000, and 28.6 per 1000, respectively. Tam *et al.* (2007) have estimated an excess risk of 60% for development of GBS following *C. jejuni* infection in the UK (Tam *et al.*, 2007). In June 2011, 26 patients in the southwestern USA were stricken with GBS after ingesting *C. jejuni*-contaminated water, showing that these sequelae can also occur in outbreaks (Jackson *et al.*, 2014). Yet, no prior studies have determined whether AR *C. jejuni* strains or AR or susceptible strains from animals are more likely to result in GBS and other long-term sequelae relative to susceptible strains.

Several forms of GBS are recognized, but the most common form occurring after *C. jejuni* infection is acute motor axonal neuropathy (AMAN) where damage to motor neurons occurs at the nodes of Ranvier. Acute motor sensory axonal neuropathy (AMSAN) can follow after AMAN leading to acute inflammatory demyelinating polyradiculoneuropathy (Oh *et al.*, 2001). *C. jejuni* infection is well known to precede the AMAN form of GBS when bacterial LOS mimics host nerve gangliosides, induces autoantibody production and causes subsequent nerve damage. This molecular mimicry between *C. jejuni* LOS structures and gangliosides found in the membranes of peripheral nerve cells is the hypothesized mechanism for GBS (van den Berg *et al.*, 2014). Upon infection lipooligosaccharides on the *C. jejuni* outer membrane elicit the production of antibodies that cross-react with gangliosides, such as GM1 and GD1a on peripheral nerves (van den Berg *et al.*, 2014; St Charles *et al.*, 2017). In human beings and other mammals, gangliosides located at or near the node of Ranvier on peripheral nerves are the target for these cross-reactive antibodies. Once these antibodies bind to the axolemma at the node, complement binds, a membrane attack complex forms, and this transmembrane channel leads to the disappearance of voltage-gated sodium channels. Subsequently, this can lead to detachment of paranodal myelin, nerve conduction failure and in some cases Wallerian degeneration (McGonigal *et al.*, 2010). Thereafter, macrophages are called in to remove myelin and axonal debris can cause more damage, but this cleanup improves the process of tissue repair (Martini and Willison, 2016). Kaida and Kusunoki (2010) have associated several *C. jejuni*-associated neurological syndromes with antibodies to gangliosides GM1, GD1a, GT1a, and GQ1b (Kaida and Kusunoki, 2010). In one study (Nachamkin *et al.*, 2002), *C. jejuni* strains associated with GBS cases had a high likelihood of having LOS resembling ganglioside GD1a, which is plentiful in the peripheral nervous system of human beings and mice (Lehmann *et al.*, 2007). Identity between *C. jejuni* LOS variants and seven different gangliosides has been demonstrated (Gilbert *et al.*, 2002). Some of these variations in LOS structure have been traced to polymorphisms in particular genes such as *cst*-II (Koga *et al.*, 2005); for example, the role of *cst*-II was verified when a *cst*-II knockout strain of *C. jejuni* was shown to be incapable of evoking anti-ganglioside antibodies in knockout mice that lack the corresponding ganglioside and thus treat it as a foreign antigen (Godschalk *et al.*, 2004).

There is considerable between-strain variation in the branched terminal oligosaccharide portion ('outer core') of *C. jejuni* LOS structure; this variation can be attributed to a variety of genetic mechanisms (Gilbert *et al.*, 2002), including lateral gene transfer (Gilbert *et al.*, 2004; Phongsisay *et al.*, 2006) and phase variation (Linton *et al.*, 2000). Phase variation has been shown (1) to occur during human infection (Prendergast *et al.*, 2004), (2) to alter invasiveness in tissue culture (Guerry *et al.*, 2002) and (3) to enhance virulence during infection in mice (Jerome *et al.*, 2011). Horizontal gene exchange, which occurs at significant rates in *C. jejuni* (Suerbaum *et al.*, 2001; Fearnhead *et al.*, 2005), has also been implicated in the generation of LOS diversity (Parker *et al.*, 2005; Phongsisay *et al.*, 2006). Extensive variation has also been demonstrated in the chromosomal region containing genes required for LOS synthesis (Gilbert *et al.*, 2002; Godschalk *et al.*, 2004; Knudsen *et al.*,

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2005; Parker *et al.*, 2005). To date, five major 'LOS classes' have been identified depending on the genetic composition of the complex LOS locus (Parker *et al.*, 2005). Godschalk *et al.* (2006) identified three GBS-associated genetic loci within the LOS region; all three encode galactosyltransferases presumably involved in the synthesis of the terminal oligosaccharide (Godschalk *et al.*, 2006).

Disease associated with *C. jejuni*: GBS in animals

Currently, rats and chickens serve as the main animal models of GBS and other peripheral neuropathies. Rats injected with myelin preparations mixed with complete Freund's adjuvant develop neurological disease and lesions. Rats show experimental autoimmune neuritis (EAN) after this immunization and exhibit mononuclear cell infiltration and demyelinated nerve fibers in peripheral nerves, e.g. the sciatic nerve (Archelos *et al.*, 1997). Rats with experimental autoimmune polyradiculo-neuropathy and nerve biopsies of GBS patients both had T cells within the epi- and perineurium, expressing a TNFα-converting enzyme likely active in these demyelinating disorders (Kurz *et al.*, 2005). However, myelin-injected rats do not model the AMAN form of GBS induced by *C. jejuni* (Nachamkin *et al.*, 1998). Chickens acquire a form of peripheral neuropathy secondary to *C. jejuni* inoculation with GBS patient strains (Li *et al.*, 1996). In chickens given a *C. jejuni* Penner serotype O:19 strain orally, 33% of the chickens became paralyzed within 12 days. In paralyzed chickens, early lesions included nodal lengthening and paranodal demyelination that was later followed by Wallerian-like degeneration and even paranodal re-myelination in some long-term survivors. Thus, chickens inoculated orally with GBS associated *C. jejuni* strains from patients can be considered a naturally occurring disease model for GBS. Work with patient samples, using rats and chickens, has helped to test the molecular mimicry hypothesis between *C. jejuni* LOS and peripheral nerve gangliosides as a mechanism for anti-ganglioside antibody induction.

C. jejuni strains from patients with GBS can produce neurological disease in mice after a single oral infection allowing the mechanisms controlling this type of autoimmunity to be studied in these models (Malik *et al.*, 2014; St Charles *et al.*, 2017). The main mechanism resulting in peripheral nerve dysfunction is molecular mimicry; some *C. jejuni* strains with sialylated outer core oligosaccharides that mimic host gangliosides (GM1/GD1a) on the peripheral nerves induced antiganglioside antibodies that attached to and damaged peripheral nerves in experimental trials. This was accompanied by peripheral neurological disease as measured on several phenotyping apparatuses. When the chloramphenicol resistant *C. jejuni* strain 260.94 was used for experimental infections, chloramphenicol treatment worsened the outcomes and intensified these autoimmune responses (St Charles *et al.*, 2017). Contrasting T cell responses were produced after *C. jejuni* infection in C57BL/6 IL-10$^{-/-}$ mice (Malik *et al.*, 2014). *C. jejuni* strains with α−2,3 sialyation in the outer core of the LOS produced Th2 responses and induction of antiganglioside antibodies against the nerves. *C. jejuni* strains without this branching produced Th1 responses and caused inflammation in the colon. These GBS mouse models are available as test systems to develop new therapeutics and to test the role of AR *C. jejuni* in driving this autoimmune disease.

Interestingly transplanted human fecal microbiota enhanced GBS autoantibody responses after *C. jejuni* infection in C57BL/6 mice. Inoculating germ-free C57BL/6 WT mice with a mixed human fecal slurry provided a murine model that stably passed its microbiota over >20 generations. ^{Hu}microbiota conferred many changes upon the WT model in contrast to previous results, which showed only colonization with no disease after *C. jejuni* challenge. When compared with ^{Conv}microbiota mice for susceptibility to *C. jejuni* enteric or GBS patient strains, infected ^{Hu}microbiota mice had (1) 10–100-fold increases in *C. jejuni* colonization of both strains, (2) pathologic change in draining lymph nodes but not colon or cecal lamina propria, (3) significantly lower Th1/Th17-dependent anti-*C. jejuni* responses, (4) significantly higher IL-4 responses at 5 but not 7 weeks post infection (PI), (5) significantly higher Th2-dependent anti-*C. jejuni* responses, and (6) significantly elevated antiganglioside autoantibodies after *C. jejuni* infection. These responses in ^{Hu}microbiota mice were correlated with a dominant *Bacteroidetes* and *Firmicutes* microbiota. Thus, this human microbiota also enhanced susceptibility to this autoimmune disease.

C. jejuni strain-dependent differences in disease outcomes

It has been suggested that colitis and GBS disease are *C. jejuni* strain dependent. Testing of many *C. jejuni* strains in the same inbred mouse model supports this hypothesis (Bell *et al.*, 2009). All *C. jejuni* strains did not produce similar disease when tested in mouse models and it has been suggested that the outcomes of hemorrhagic colitis and autoimmunity such as GBS are strain dependent (Malik *et al.*, 2014). C57BL/6 IL-10$^{-/-}$ mice infected with isolates from patients with colitis had significantly upregulated type 1 and 17 but not type 2 cytokines in the colon coincident with infiltration of phagocytes, T cells and innate lymphoid cells (ILCs). Both ILC and T cells contributed to the interferon-c, IL-17, and IL-22 upregulation, but in a time- and organ-specific manner. However, T cells were necessary for colitis as mice depleted of Thy-1þ cells were protected while neither Rag1-/- nor IL-10R blocked Rag1-/- mice developed colitis after infection. Depleting IFN-c, IL-17, or both significantly decreased colitis and drove colonic responses toward type 2 cytokine and antibody induction. However, *C. jejuni* strains from GBS patients induced mild colitis with mixed cytokine profiles associated with blunted type 1/17 but enhanced type 2 responses. It was only the type 2 antibody isotypes that cross-reacted with peripheral nerve gangliosides producing autoimmunity. Thus, contrasting T-cell responses contributed to either colitis or autoimmunity in C57BL/6 IL-10$^{-/-}$ mice (Malik *et al.*, 2014). This reflects the roles of Th1 responses in killing intracellular pathogens and of Th2 responses in the induction of autoimmunity (Nurieva and Chung, 2010).

Arguments against strict strain dependent disease pathotypes include, (1) varied animal reservoirs such as poultry, swine,

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cattle, and fecal contaminated water are the most common sources of human infections, and (2) in one outbreak *C. jejuni* isolates from patients with colitis and GBS were genetically identical. These outcomes may be explained by recognizing that not all *C. jejuni* strains have ganglioside mimics on their surface, but have other glycans (Nachamkin *et al.*, 2002). Moreover, many individuals infected with *C. jejuni* bearing self-glycans will maintain tolerance and elicit protective responses against other surface molecules of the bacterium (Willison *et al.*, 2016), and the mechanisms controlling B-cell tolerance to T-cell-independent antigens are largely unknown.

C. jejuni within-host adaptation can enhance disease

C. jejuni's genome is not static during *in vivo* passage (Wassenaar *et al.*, 1998; Nuijten *et al.*, 2000; de Boer *et al.*, 2002; Jerome *et al.*, 2011; Kim *et al.*, 2012; Kivistö *et al.*, 2014). A significant proportion of this genomic variation occurs in virulence-associated genes that are involved in the synthesis of antigenic structures including the LOS, flagella, and capsule that are involved in triggering immune responses and potentially aiding in immune evasion (Jerome *et al.*, 2011; Kivistö *et al.*, 2014). Some genomic variants have direct links to biological outcomes, such as increased motility; thus *C. jejuni* adaptation does influence infection outcomes.

The first evidence for *C. jejuni* adaptation *in vivo* came from variability in pulse-field gel electrophoresis banding patterns following passage in chickens, where analysis of initially clonal isolates of *C. jejuni* revealed multiple banding patterns in recovered isolates, providing evidence that large-scale genomic rearrangements occurred during *in vivo* passage (Wassenaar *et al.*, 1998). Concurrently, Parkhill *et al.*, 2000 identified hypervariable regions in the *C. jejuni* 11168 genome consisting of homopolymeric tracts of nucleotides (Parkhill *et al.*, 2000). Since this discovery, our laboratory has shown that insertions or deletions in homopolymeric tracts of nucleotides allow *C. jejuni* 11168 to rapidly adapt during passage in mice (Jerome *et al.*, 2011). *C. jejuni* farm isolates also contained variants in homopolymeric tracts (Kivistö *et al.*, 2014). In both cases, the majority of variants in homopolymeric tracts were found in the LOS, capsular, and flagellar genes. Collectively, these homopolymeric tracts were called contingency loci as they have higher rates of mutation than the rest of the genome. Mutations in contingency loci contribute to phase variation: the ability to turn gene expression on or off (Moxon *et al.*, 1994); phase variation may directly impact pathogenesis by altering the expression of virulence factors including LOS, capsule, and flagella (Jerome *et al.*, 2011).

Variation in homopolymeric tract length can result in observable biological outcomes. Notably, the passage of *C. jejuni in vivo* led to the presence of antigenic ganglioside mimics on the LOS of *C. jejuni* 81–176 that initially lacked any ganglioside mimics (Prendergast *et al.*, 2004). Site-directed mutagenesis of homopolymeric tracts in the *cgt*A gene (N-acetyl-galactosaminyl transferase) in *C. jejuni* 81–176 shifted the ratio of GM2 and GM3 ganglioside mimics and enhanced the invasiveness of the *C. jejuni cgt*A mutant compared with the WT strain (Guerry *et al.*, 2002). The host environmental cues that drive evolutionary selection for phase variation are unknown, but it is known that this process allows for rapid adaptation to novel environments, increased diversity, and evasion of the host immune system (van der Woude and Baumler, 2004; Jerome *et al.*, 2011). Slipped-strand mutagenesis (Moxon *et al.*, 1994; Zhou *et al.*, 2014) and the absence of several homologs of *E. coli* DNA repair genes contribute to the high incidence of phase variation in *C. jejuni* (van der Woude and Baumler, 2004). To our knowledge little has been done to understand the role of antibiotic selection on phase variability.

Other mechanisms of *C. jejuni* adaptation that affects disease

C. jejuni has other mechanisms of adaptation to novel environments in addition to variation in homopolymeric tracts. Several recent studies have demonstrated that single nucleotide variants outside of homopolymeric tracts contribute genetic diversity to *C. jejuni* strains (Cagliero *et al.*, 2006; Mohawk *et al.*, 2014; Thomas *et al.*, 2014). In addition, phenotypic adaptation in *C. jejuni* has also been observed. *In vivo* passage conferred increased motility in mice and rabbits (Caldwell *et al.*, 1985; Jones *et al.*, 2004), although this is not surprising because motility is a major factor in *C. jejuni* colonization (Nachamkin *et al.*, 1993; Wassenaar *et al.*, 1993; Yuki *et al.*, 1993). It has also been shown that *C. jejuni* virulence increases following passage both in similar (Bell *et al.*, 2009) or divergent hosts (Kim *et al.*, 2012), which may result from enhanced colonization potential (up to 10,000-fold increase) (Cawthraw *et al.*, 1996), alterations in virulence-associated gene expression (Bell *et al.*, 2013a), or the presence of antigenic stimuli based on modifications of surface structures of *C. jejuni* (Prendergast *et al.*, 2004). Intragenomic recombination can also occur during passage and even restore motility in non-motile *flaA* mutants (Nuijten *et al.*, 2000) or enhance phage resistance (Scott *et al.*, 2007). Intergenomic recombination is possible, but current data are limited to recombination between *C. jejuni* strains with highly similar gene content (de Boer *et al.*, 2002). Together, these results confirm the plasticity of the *C. jejuni* genome while establishing that adaptation of *C. jejuni* often affects loci with the potential to modulate host immune responses.

Conclusions

These results from people and animals infected with *C. jejuni* demonstrate that there are many factors affecting the development of the disease. They also show the spectrum of diseases produced by *C. jejuni* strains and the plasticity of *C. jejuni* strains initiating these outcomes. Furthermore, the gut microbiome plays an important role in enhancing colonization resistance to *C. jejuni*. It appears that AR *C. jejuni* may cause more severe disease, but this must be confirmed and the mechanisms delineated to fully understand the risks associated with this growing problem. More work is needed to understand the mechanisms underlying these interacting factors resulting in *C. jejuni* disease.

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