

Lack of Association between the Presence of the pVir Plasmid and Bloody Diarrhea in *Campylobacter jejuni* Enteritis

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The main mechanisms by which *Campylobacter jejuni* causes diarrhea are unknown. In contrast to a recent communication, we report here the absence of an association with the plasmid pVir in patients infected with *C. jejuni* who developed bloody diarrhea in The Netherlands, and we suggest a role for other virulence determinants.

Campylobacter jejuni is the leading cause of human bacterial gastroenteritis worldwide. A great variety of clinical symptoms are observed in patients infected with *C. jejuni*, ranging from asymptomatic carriage and mild watery diarrhea to severe and bloody diarrhea with fever. Disease complications include bacteremia, reactive arthritis, and acute postinfectious neuropathies: the Guillain-Barré syndrome (GBS) and the Miller-Fisher syndrome (MFS).

The virulence factors involved in the pathogenesis of *C. jejuni* diarrhea are still poorly characterized. Various mechanisms have been reported to be involved, including adherence, cellular invasion, and toxin production. Motility and multiple adhesins appear to play a role in intestinal adherence and colonization (6, 9). In addition, lipooligosaccharide structures have been found to be involved in the pathogenesis of postinfectious neuropathy by molecular mimicry with human gangliosides (5). A significant proportion of *C. jejuni* harbors plasmids, and the contribution of plasmids in the pathogenesis and antimicrobial resistance of *Campylobacter* infections has been studied since the early 1980s (7). The plasmid pVir has been implicated in the virulence of *C. jejuni* (1). pVir contains genes for homologues of the Com and Vir proteins, which are presumably involved in DNA uptake or protein transport via a putative bacterial type IV secretion machinery (1). More recently, Tracz et al. (8) identified pVir in 17 of 104 (17%) clinical *C. jejuni* isolates and found that isolates containing pVir were associated with the presence of a tetracycline resistance plasmid. In addition, these authors report a significant association of the presence of pVir with bloody diarrhea and suggest an important role of pVir in the pathogenesis of more severe invasive *Campylobacter* infections.

We detected pVir and the *tet(O)* gene in *C. jejuni* strains, isolated from 125 well-characterized community-based Dutch patients and analyzed the association with bloody diarrhea (3). In addition, since preliminary studies in our laboratory (data not shown) indicated that GBS/MFS-related *C. jejuni* are more invasive in vitro, we wondered whether the presence of pVir

was elevated among a particular set of 21 *C. jejuni* strains isolated from GBS/MFS patients in The Netherlands.

Plasmid and chromosomal DNA was isolated by using QIAamp DNA Minikit (QIAGEN). pVir and *tet(O)* were detected by PCR according to the protocol described by Tracz et al. (8). *C. jejuni* 81-176 was used as a positive control. Tetracycline MICs were determined by using the Etest method on Mueller-Hinton agar supplemented with 5% sheep blood. *C. jejuni* ATCC 33560 was used as quality control strain.

pVir was detected in 4 of 125 (3%) enteritis strains and in 1 of 17 (6%) of the GBS-related strains and was not detected in the four MFS-related strains. *tet(O)* was found in 41 of 125 (32%) enteritis strains, in 4 of 17 (24%) of the GBS-related strains, and in 1 of 4 MFS-related strains (25%). All five GBS/MFS-related strains that contained *tet(O)* had MICs for tetracycline of >256 mg/liter. The strains that did not harbor the *tet(O)* gene had MICs for tetracycline that ranged from 0.064 to 0.5 mg/liter. The prevalence of *tet(O)* among pVir-negative strains was 33%. Bloody diarrhea was reported in 48 of the 125 patients. Only 1 of the 48 patients was infected by a pVir-positive *C. jejuni* strain.

Our data point toward a remarkably low prevalence of pVir in *Campylobacter* strains in The Netherlands in contrast to a recent report from Canada (8). Furthermore, no significant difference was observed in the prevalence of pVir in 125 enteritis strains compared to Dutch GBS and MFS strains, although the number of GBS/MFS strains was small since these isolates are infrequently isolated. The absence of an association between the presence of pVir and bloody stools suggests that other virulence factors may be involved in the development of bloody diarrhea. A suggestion in this direction was recently made by Champion et al. (2), who proposed a serine protease encoded by *Cj1365* as a virulence factor involved in the development of bloody diarrhea. Previously, serine proteases have been described in *Enterobacteriaceae* and are thought to be involved in the development of bloody diarrhea caused by *Escherichia coli* (4).

One must emphasize that the loss of plasmids during subculture cannot be excluded, although observations on the stability of pVir and the tetracycline resistance plasmid in strain 81-176 do suggest that this appears to be an infrequent event (1). Alternatively, if we compare the data of Tracz et al. and ours, one wonders whether patient and geographical charac-

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teristics may explain the observed differences. Our patient group comprised 125 well-characterized, community-based Dutch patients, none of whom required hospitalization during the course of the disease. Differences in disease severity or geographical locale may eventually parallel the great variety in prevalence of pVir in *Campylobacter* isolates. Thus, our data add to those of Tracz et al. and further strengthen their conclusions that more studies are needed to assess the association of pVir and other virulence markers among the widening spectrum of *C. jejuni* that cause disease in humans.

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