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Campylobacter upsaliensis: Waiting in the Wings

BILLY BOURKE,[1]* VOON LOONG CHAN,[2] AND PHILIP SHERMAN[3]

Department of Paediatrics, University College Dublin, The Children's Research Institute, Our Lady's Hospital for Sick Children, Dublin, Ireland,[1] and Department of Pediatrics, Division of Gastroenterology, The Hospital for Sick Children,[3] and Department of Medical Genetics and Microbiology, University of Toronto,[2] Toronto, Ontario, Canada

INTRODUCTION

The identification of novel pathogens is a perennial concern among microbiologists and others interested in the study of infectious diseases. In recent years, many novel gastrointestinal pathogens have come to attention. Some, such as enterohemorrhagic *Escherichia coli* (50), *Cryptosporidium parvum* (22), and *Helicobacter pylori* (8), have achieved widespread and fairly rapid recognition. The contribution of other microbes to human enteric diseases has been greeted with less certainty. *Campylobacter upsaliensis* is an organism which, despite consistently convincing epidemiological evidence supporting its role as a human enteric pathogen, languishes in the latter category. *C. upsaliensis* is rarely isolated in clinical laboratories and therefore is little known among clinicians. This underrecognition is due, at least in part, to the fact that *C. upsaliensis* is sensitive to the antibiotics routinely used in *Campylobacter* selective media. Heightened awareness and improved isolation techniques will undoubtedly yield higher *C. upsaliensis* isolation rates and help to bring this enteric pathogen to center stage in the realm of human enteric pathogens.

C. UPSALIENSIS AS A HUMAN PATHOGEN

In 1983, Sandstedt et al. (78) reported the presence of a novel catalase-negative *Campylobacter* sp. isolated frequently from the feces of dogs attending an animal clinic in Uppsala, Sweden. These organisms were originally referred to as the catalase-negative/catalase weak (CNW) group. In the year following their initial description, two reports described the isolation of CNW organisms from canine feces (24, 34). In 1985, Steele et al. (84) provided the first description of CNW organisms in human stools. On the basis of DNA homology studies, these organisms were shown to form a separate *Campylobacter* species, which was later named *Campylobacter upsaliensis* after the city in which it was first described (3). Since then, reports have emerged worldwide implicating *C. upsaliensis* as a human bacterial enteropathogen (23, 41, 55, 58, 61, 70, 88, 93). In fact, a number of investigators (41, 55, 58), have isolated *C. upsaliensis* from stools more frequently than *C. coli*, an acknowledged human enteropathogen.

C. upsaliensis is associated with acute self-limiting diarrhea but has also been isolated in the setting of chronic and recurrent diarrhea (39). Weight loss accompanying *C. upsaliensis*-related diarrhea also has been described (61). Moreover, *C. upsaliensis* can cause bacteremia in debilitated and immunocompromised patients (21) and has been associated with extraintestinal infections (33), spontaneous human abortion (44), hemolytic-uremic syndrome (17), and Guillain-Barré syndrome (37, 47).

Preliminary investigations into the virulence mechanisms of this organism have appeared only recently (detailed below). Koch's postulates have not yet been fulfilled for this organism. Nevertheless, existing clinical and epidemiological data offer compelling evidence supporting the importance of *C. upsaliensis* as a human enteropathogen.

TAXONOMY

DNA hybridization studies performed by Sandstedt et al. (78) on the first reported isolates of *C. upsaliensis* indicate that

* Corresponding author. Mailing address: Department of Paediatrics, University College Dublin, Children's Research Centre, Our Lady's Hospital for Sick Children, Crumlin, Dublin 12, Ireland. Phone: 353-1-455 6901. Fax: 353-1-455 5307. E-mail: bbourke@crumlin.ucd.ie.

TABLE 1. Phenotypic characteristics of *Campylobacter* species[a]

Species	Characteristic[b]							G+C content (mol%)
	Catalase production	Nitrate reduction	Indoxyl Acetate	Hippurate hydrolysis	Tolerance to nalidixic acid	H2S production on TSI agar	Growth on potato starch	
C. upsaliensis	−/w	+	+	−	−	−	+	33–36
C. jejuni	+/v	+	+	+	−	−	n	30–32
C. coli	+	+	+	−	−	v	n	31–33
C. lari	+	+	−	−	+	−	n	31–33
C. fetus	+	+	−	−	+	−	n	33–34
C. hyointestinalis	+	+	−	−	+	+	n	35–36
C. concisus	−	+	−	−	+	+	n	38–39
C. mucosalis	−	−	−	−	+	+	n	38–39
C. sputorum	−	+	−	−	+	+	n	31–33
C. helveticus	−	+	+	−	−	−	−	34
C. curvus	−	+	+	−	+	+	n	43–47
C. rectus	−	+	+	−	+	+	n	42–46
C. showae	+	+	+	−	−	+	n	44–46
C. hyoilei	+	+	n	−	−	+	n	35
C. gracilis	−	+	−	−	+	+	n	44–46

[a] Adapted from reference 10.
[b] Test results: +, positive reaction; −, negative reaction; w, weak reaction; v, variable reaction; n, not known.

they belong to a homogeneous group (80 to 96% intragroup relatedness and 40% relatedness to other thermotolerant *Campylobacter* spp.). Following this initial report, these organisms were referred to as the CNW group. The first human isolates of this organism (84) were confirmed as *C. upsaliensis* by using DNA probes which demonstrated their similarity to the CNW organisms originally isolated by Sandstedt et al. (78). In 1991 Sandstedt and Ursing (77) proposed the name *Campylobacter upsaliensis* for the CNW group; the name was validated in 1991 (3).

The G+C content of *C. upsaliensis* varies from 32.8 to 35.8 mol% (78, 91). This is somewhat greater than the G+C content of *C. jejuni* (30 to 32.6 mol%) and *C. coli* (30.8 to 32.5 mol%) but is similar to that described for other *Campylobacter* species including *C. fetus* (33.3 to 34.5 mol%) and *C. hyointestinalis* (33.6 to 35.2 mol%) (91). DNA-rRNA hybridization and immunotyping techniques (91) confirm the phylogenetic similarity of *C. upsaliensis* to other members of the *Campylobacter* genus.

A methyl-substituted menaquinone which appears unique to *Campylobacter* species is also present in *C. upsaliensis* (66). This menaquinone species, known as menaquinone-6, is a member of the isoprenoid quinone family present in the plasma membrane of bacteria and functions in electron transport (65).

LABORATORY DETECTION

C. upsaliensis is a microaerophilic, thermotolerant, motile, curved, gram-negative rod. The organism has a single polar or bipolar flagellum and exhibits the darting movements characteristic of *Campylobacter* spp. under phase-contrast microscopy. It forms smooth, pinpoint, greyish or translucent colonies on blood agar plates. Swarming may be observed when the organism is grown on moist media (77). Growth in broth requires supplementation with sheep blood (70) or fetal calf serum (9). The organisms are 0.3 to 0.4 μm wide and 1.2 to 3 μm long. On exposure to air, coccoid forms may appear.

This bacterium is oxidase positive, nitrate positive, and hippurate negative (71) (Table 1). *C. upsaliensis* is sensitive to nalidixic acid and, usually, to cephalothin. The presence of these antibiotics in the selective media generally used for the isolation of *Campylobacter* species (e.g., Skirrow's medium) may well account for the suboptimal identification of *C. upsaliensis* in clinical specimens at most centers (4, 41, 70).

Successful isolation of *C. upsaliensis* from stool specimens currently relies on the use of a filtration method (41, 58, 64). This method enriches *Campylobacter*-infected fecal specimens and thereby helps increase the yield when grown on solid media (83). This is accomplished by using a filter with a pore size sufficiently large to permit passage of the small campylobacter organisms but small enough to exclude larger fecal contaminants. Goossens et al. (41) found that a filter system with a pore size of 0.45 μm resulted in less contamination than did one with 0.65-μm filters. However, the authors also pointed out that bacterial concentrations of less than 10^5 CFU per g of feces could not be detected by the filter method. Therefore, a more sensitive detection method (such as a specific genetic probe) might yield even higher isolation rates for *C. upsaliensis* in clinical specimens.

In addition to a lack of sensitivity, the use of the filtration method is more cumbersome than the use of selective agar media. Therefore, the development of selective media for the successful isolation of *C. upsaliensis* from clinical samples would be beneficial. Walmsley and Karmali (93) successfully used a selective medium containing cefoperazone (32 μg/ml), vancomycin (20 μg/ml), and cyclohexamide (100 μg/ml) without filtration to isolate *C. upsaliensis* from the stools of six pediatric patients. However, they did not directly compare the utility of this technique with that of the filtration method.

Aspinall et al. (4, 5) described the use of a new selective medium for the isolation of thermophilic campylobacters. A blood-free medium containing cefoperazone (8 μg/ml) amphotericin (10 μg/ml), and teicoplanin (4 μg/ml) (CAT) was compared with a commercially available *Campylobacter* selective medium containing cefperazone (32 μg/ml) and amphotericin (10 μg/ml) in a blood-free selective agar base (modified CCDA) and with a filtration method. CCDA and CAT demonstrated comparable isolation rates for campylobacters other than *C. upsaliensis*. Of significance, the CAT medium correctly isolated 84% of *C. upsaliensis* isolates from spiked fecal samples (comparable to 90% sensitivity for the filtration method in the same study), while the modified CCDA isolated only 29% of isolates. Further investigations comparing this technique with the filtration method are now required.

C. upsaliensis can be differentiated from *C. jejuni* by its lack of catalase activity and an inability to hydrolyze hippurate (77). It can be distinguished from *C. coli* and *C. hyoilei* by its lack of catalase activity and from *C. lari*, *C. fetus* and *C. hyointestinalis* by its lack of catalase activity and its sensitivity to nalidixic acid (2, 77) (Table 1). *C. upsaliensis* can be differentiated from the catalase-negative campylobacters (i.e., *C. sputorum*, *C. concisus*, *C. curvus*, *C. rectus*, and *C. mucosalis*) by its lack of hydrogen sulfide production on triple sugar iron (71, 77) and from *C. gracilis* by its positive oxidase test (86). The recently described *C. helveticus* (81) has many phenotypic and biochemical similarities to *C. upsaliensis*. Therefore, it may prove difficult to differentiate between these two organisms by conventional biochemical laboratory testing. However, the colony morphology of *C. upsaliensis* is distinctive (81). Pinpoint, grey colonies are typical of *C. upsaliensis*, whereas colonies of *C. helveticus* are flat and smooth with a watery, spreading appearance on blood agar. In addition, *C. helveticus* can be differentiated from *C. upsaliensis* by both its inability to reduce selenite and its lack of growth on potato starch medium (81). In conclusion, the most useful biochemical tests for the identification of *C. upsaliensis* in the clinical microbiology laboratory include those for catalase production, hippurate hydrolysis, nitrate reduction, oxidase activity, H_2S production on triple sugar iron agar, and sensitivity to nalidixic acid (77).

A number of investigators have applied molecular techniques to directly identify enteric campylobacters from stools (27, 35, 68). Eyers et al. (27) identified regions of 23S rRNA genes specific for thermophilic *Campylobacter* strains, including *C. upsaliensis*. By designing oligonucleotide primers corresponding to these specific 23S rRNA regions, they were able to distinguish thermophilic *Campylobacter* strains from other fecal microorganisms and also to discriminate between individual *Campylobacter* species. Because isolation and accurate identification of *Campylobacter* species from fecal specimens by using standard phenotypic testing is problematic (68), PCR-based assays and other molecular methods soon may become standard identification techniques for these organisms.

Typing Methods

Since *C. upsaliensis* has only recently been recognized as a human pathogen, the development of applicable typing methods for this organism is still at an evolutionary stage. In two reports from South Africa (23, 56), few *C. upsaliensis* strains were typable by the lipopolysaccharide (heat-stable antigen) method of Penner et al. (72). A serotyping method based on the detection of heat-labile antigens was recently described by Lior and Woodward (59). This serotyping scheme recognizes seven serogroups, with groups 1, 2, 3, and 5 being the most common among human isolates of *C. upsaliensis*. No cross-reactivity is observed with *C. jejuni*, *C. coli*, or *C. lari* immune sera.

A number of typing methods have been compared in investigating a *C. upsaliensis* outbreak in four day care centres in Brussels in 1991 (36, 39). Thirty-four isolates were characterized by plasmid analysis, DNA restriction enzyme analysis, whole-cell protein analysis, restriction fragment length polymorphism (RFLP), and PCR typing. On the basis of the PCR and RFLP results, the outbreak was attributed to two closely related clonal variants of *C. upsaliensis*. However, no specific typing method examined was identified as ideal for widespread epidemiological typing studies of this organism.

The observation of genotypic heterogeneity among strains of *C. upsaliensis* by RFLP (69) and pulsed-field gel electrophoresis (11) indicates the likely utility of molecular techniques for epidemiological purposes. Recently, we have shown little similarity across a range of *C. upsaliensis* strains (11) (Fig. 1). In that study, 19 *C. upsaliensis* strains obtained from the Laboratory Centre for Disease Control (Ottawa, Canada) and the type strain (ATCC 43954) were analyzed by pulsed-field gel electrophoresis. These *C. upsaliensis* strains were geographically diverse in origin and comprised both canine and human isolates. In contrast to similar studies on other campylobacters, genomic diversity was evident among *C. upsaliensis* isolates when a range of different rare-cutting restriction enzymes was used. These typing methods now warrant further evaluation in the setting of outbreaks of *C. upsaliensis* infection.

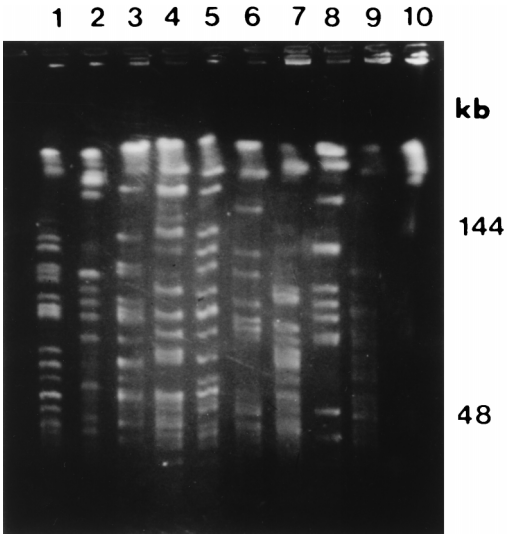

FIG. 1. *Xho*I-generated macrorestriction patterns demonstrating genotypic heterogeneity among *C. upsaliensis* isolates. Reprinted from reference 11 with permission of the publisher.

EPIDEMIOLOGY

C. upsaliensis has been isolated from patients on four continents. However, it is sensitive to antibiotics, such as cephalothin, frequently used in selective media used for isolation of enteric campylobacters (41, 70, 93). Therefore, the contribution of *C. upsaliensis* to diarrheal disease and other human illnesses is difficult to determine from the present data.

Animal Reservoirs and Transmission

The observation that animals represent a reservoir for human infection with *C. jejuni* and *C. coli* (6, 7, 79) may also hold true for *C. upsaliensis*. Most of the isolates in the initial report by Sandstedt et al. (78) were from dogs with diarrhea. Davies et al. (24) described a *C. upsaliensis* isolate from a dog with chronic diarrhea. However, some isolates were from asymptomatic animals (28, 78), including all of the feline isolates identified in studies by Fox et al. (32) and Moreno et al. (64).

Most animal *Campylobacter* isolates were previously reported to be either *C. jejuni* or *C. coli* (6, 80). However, Moreno et al. (64), who isolated *Campylobacter* strains by a filtration method, documented extremely high rates of carriage of *C. upsaliensis* (66%) among domestic and laboratory cats. In contrast, only 3% of this animal population harbored *C. jejuni* and none carried *C. coli*. In a study of dogs, Sandstedt et al. (78) found that *C. upsaliensis* accounted for 63 (64%) of 98 strains of *Campylobacter* identified over 2 years. *C. upsaliensis*

was also more commonly isolated than other campylobacters from canine feces in a study from Italy (28). Since the rate of *Campylobacter* carriage in dogs may be up to 75% (14, 45, 74), *C. upsaliensis* appears to be a common, albeit frequently unrecognized, environmental organism.

Evidence to support the transmission of *C. upsaliensis* infection from animals to humans comes from two reports implicating *C. upsaliensis* as a cause of both human enteritis and spontaneous abortion (40, 44). Goossens et al. (40) demonstrated *C. upsaliensis* in stools obtained from a 53-year-old man with acute onset of pyrexia and bloody diarrhea. A *C. upsaliensis* strain, apparently of some considerable similarity to that obtained from this patient, was also isolated from the patient's asymptomatic dog. Gurgan and Diker (44) reported finding *C. upsaliensis* in cultures of blood and fetoplacental material from a woman suffering a spontaneous abortion at 18 weeks gestation. *C. upsaliensis* was also isolated from her asymptomatic household cat; analysis of protein profiles confirmed strong similarity between the human and feline bacterial isolates. Nonetheless, the transmission of *C. upsaliensis* from animals to humans remains to be conclusively proven. A recent study by Stanley et al. provides evidence against dog-to-human transmission of *C. upsaliensis* (82). In this study *C. upsaliensis* isolates from humans had a conserved 16S rRNA ribotype, which is not found among canine strains. Data from this study suggest that specific clones within *C. upsaliensis* are responsible for human disease.

Indirect evidence supporting the possibility of person-to-person spread of *C. upsaliensis* comes from two studies. Walmsley and Karmali (93) isolated *C. upsaliensis* from two asymptomatic patients in Toronto who had shared a hospital room (fecal cultures were performed because of contact with another child with a fecal *Salmonella* isolate). More recently, Goossens et al. (39) identified 34 children with *C. upsaliensis* in four day care centers in Brussels, Belgium. On the basis of multiple typing methods, it was demonstrated that the outbreaks of *C. upsaliensis* infection in three of the four centers were due to the same organism. Furthermore, the *C. upsaliensis* strain responsible for these outbreaks was closely related to the strain isolated from an out break in the fourth day care center.

Frequency of Isolation from Human Feces

C. upsaliensis contributes significantly to the total *Campylobacter* isolation rates in diarrhea. Over a period of 3 years, Goosens et al. (41) identified *C. upsaliensis* in 99 of a total of 15,185 stool specimens (0.65%); this represented 12% of all *Campylobacter* isolates in the study. Megraud and Bonnet (61) found *C. upsaliensis* in 9% of pediatric *Campylobacter* isolates. In Australia, Steele et al. (84) found a total of 104 *Campylobacter* isolates by a filter technique; 9 (8.7%) of these isolates were *C. upsaliensis* strains. Of note, *C. upsaliensis* was a major isolate in a subgroup of 217 stool specimens taken from children aged 3 years or younger, in which it accounted for 8 (26.7%) of 30 *Campylobacter* isolates. *C. upsaliensis* consistently accounts for over 20% of all *Campylobacter* isolates at The Red Cross War Memorial Children's Hospital in South Africa (55). Lindblom et al. (58) recently showed that *C. upsaliensis* accounts for 18% of all *Campylobacter* isolates among children in Göteborg, Sweden. In this study, *C. upsaliensis* was observed six times more often in the stools of pediatric patients than was *C. coli*. Although it is difficult to extrapolate with confidence from these data to other populations, it appears that *C. upsaliensis* may account for over 10% of all fecal *Campylobacter* isolates. This figure may be closer to 20% among infants and young children.

Goossens et al. (41) reported that *C. upsaliensis* may also be a cause of traveller's diarrhea. The population studied in this Belgian investigation included a large number of immigrants, mostly from Morocco. Many immigrants having travelled to their country of origin in the early summer to visit family would have been exposed to new members of the microbiological flora, often in unsanitary conditions. The authors suggested that higher isolation rates for *C. upsaliensis* infection noted in the late summer months might be explained by the return of these immigrants with newly acquired *C. upsaliensis* infections. Otherwise, there is a paucity of data concerning seasonal, demographic patterns and risk factors for acquisition of *C. upsaliensis* infection.

CLINICAL EXPERIENCE

Since the first description of human *C. upsaliensis* isolates from Australia (84), the organism has been found in human feces and blood cultures in France (61), South Africa (55, 56), Canada (88), the United States, (70), Belgium (38, 41), the United Kingdom (4), Austria (46), and Sweden (58). *C. upsaliensis* has also been identified in blood cultures obtained from febrile patients with and without immunodeficiency (21, 70).

Clinical Features Associated with Infection

Goossens et al. (41) evaluated stool specimens for the presence of *C. upsaliensis* in a large population in Belgium. By using a filtration method, *C. upsaliensis* was identified in 99 of a total of 15,185 specimens examined. Of the 77 patients (73 children and 4 adults) for whom clinical information was available, 92% had diarrhea. Typically the onset of illness was sudden and the symptoms were relatively mild, lasting for less than a week. Gross or occult blood was present in only 25% of samples, and leukocytes were detected in fecal smears in fewer than 20% of the patients.

A later study by the same group in Belgium documents person-to-person transmission of *C. upsaliensis* infection in day care centers in Brussels (39). Although few clinical details were provided in this paper, apparently the disease was mild and self-limiting in most cases. However, some children did experience chronic or recurrent diarrhea.

In a study in Toronto (93), *C. upsaliensis* was identified in stools from six children whose age ranged from 3.5 to 36 months. Three of the children had watery stools, vomiting, and anorexia at the time of the positive isolate. The illness was self-limiting in two of the children, but the third child had a prolonged illness, with diarrhea lasting for 3 weeks. One of the other children had fever of unknown origin (without diarrhea), and the remaining two children were completely asymptomatic. No other bacterial pathogens were isolated from the stools of these patients.

In another study in Canada, Taylor et al. (88) described seven *C. upsaliensis* isolates, five of which were from children less than 2 years old. All seven strains were isolated from patients with diarrhea. However, no other clinical description was provided. Megraud and Bonnet (61) identified *C. upsaliensis* in stool samples from seven French children, six of whom were younger than 10 months. All seven children had gastrointestinal disturbance, with the major features including diarrhea, vomiting, and fever. In three children, the illness lasted for more than 7 days. As in the majority of reports, no other stool pathogens were isolated from these children. The

presence or absence of blood or pus cells in the stools of these patients was not discussed.

The clinical features of 11 patients (3 children and 8 adults) with *C. upsaliensis* in blood or stool specimens were described by Patton et al. (70). The stool specimens were isolated from three patients with vomiting, diarrhea, and fever. Two of these patients had abdominal pain, but only one had bloody diarrhea. One of the three patients with *C. upsaliensis* in the stool was leukopenic while on anticancer chemotherapy; the other two had been previously well. Eight patients ranging from 6 months to 83 years of age had *C. upsaliensis* in blood culture specimens. Six of these had an underlying medical condition which might have predisposed them to an opportunistic infection; no predisposing condition was identified in the other two patients. Three of the patients with positive blood culture results had diarrhea, and two others had undergone abdominal surgery. Otherwise, no specific source of infection with *C. upsaliensis* could be identified. This study offers strong supportive evidence that *C. upsaliensis* is a human pathogen causing enteritis and bacteremia in normal hosts and opportunistic infection in immunocompromised individuals.

C. upsaliensis bacteremia in the setting of immunodeficiency was also described in a case report by Chusid et al. (21). *C. upsaliensis* bacteremia was identified in a 16-year-old boy with acquired hypogammaglobulinemia secondary to nephrotic syndrome. A similar association between hypogammaglobulinemia and recurrent bacteremia due to *C. jejuni* infection has been noted (92).

Lastovica et al. (56) described the isolation of *C. upsaliensis* in cultures of 17 blood samples (from 16 pediatric patients) of a total of 28,576 blood cultures examined. The average age of the patients was 15.5 months, and all had an underlying illness (including eight with acute enteritis and six with kwashiorkor [protein-predominant malnutrition]). Although the presence of enteritis in these patients suggests an intestinal source of the *C. upsaliensis* bacteremia, the stools of these patients were not investigated for the presence of *C. upsaliensis*.

Recently, *C. upsaliensis* has been associated with hemolytic-uremic syndrome. Carter and Cimolai described a 14-year-old with abdominal pain and profuse watery diarrhea who developed microscopic hematuria, thrombocytopenia, and acute renal failure (17). A renal biopsy confirmed hemolytic-uremic syndrome, and *C. upsaliensis* was isolated from stools. No other pathogen, including sorbitol-negative *Escherichia coli*, was isolated, and PCR failed to amplify verotoxin genes from stools.

From these reports, it appears that *C. upsaliensis* is associated with a similar disease spectrum to that described previously for *C. jejuni* (71). An acute, self-limited diarrheal illness is the most usual presentation, while fever, vomiting, and abdominal pain are inconsistent features. In a minority of patients, blood or leukocytes are present in the stools. Asymptomatic infection may also occur. Young infants and children may be more frequently affected than adults. Infection in the pediatric age group may be associated with protracted diarrhea. Bacteremia occurs primarily in debilitated and immunocompromised individuals.

C. upsaliensis has also been isolated from other extraintestinal sites including a breast abscess (33) and fetoplacental material from a spontaneous human abortion (44). In addition, *C. upsaliensis* recently has been reported in association with postinfectious polyneuropathy (37, 47). Ho et al. documented an acute motor axonal neuropathy pattern of Guillain-Barré syndrome in a 64-year-old woman with antecedant *C. upsaliensis*-related diarrhea (47). Anti-*C. upsaliensis* lipopolysaccharide antibodies were present in her serum, including anti-ganglioside GM1 antibodies. Antibodies to GM1-like epitopes on lipopolysaccharides of *C. jejuni* strains associated with Guillain-Barré syndrome are thought to cross-react with the patient's myelin sheath to produce nerve damage in this disorder. In another report, *C. upsaliensis* was isolated from a 4-year-old child in South Africa who was undergoing prolonged ventilation because of Guillain-Barré syndrome (37). To date, there has been no reported association of *C. upsaliensis* with Miller-Fischer syndrome, another polyneuropathy strongly associated with antecedent *C. jejuni* infection (75).

Immunological responses to *C. upsaliensis* following infection offer additional, indirect evidence for the pathogenicity of the organism in humans. Megraud and Bonnet (61) examined convalescent-phase serum samples from five patients who excreted *C. upsaliensis* in their stools. A complement fixation test showed that antibody titers against *C. upsaliensis* were significantly elevated in serum in three of the five patients. Goossens et al. (39) demonstrated specific immunoglobulin G (IgG), IgM, and IgA antibodies in serum in 21 of 26 affected children in a multicenter outbreak of *C. upsaliensis* infection involving four day care centers. Patton et al. (70) assayed *C. upsaliensis* isolates for their susceptibility to complement-mediated bactericidal activity and found that each of four stool isolates were susceptible to bactericidal activity present in normal human serum whereas seven of eight *C. upsaliensis* blood isolates displayed resistance. This indicates that the immunological responses of the host could play an important role in modulating the pathogenic effects of the organism; i.e., more invasive infections (e.g., bacteremia) occur only if host immune defenses mount a suboptimal response to an infecting strain.

Antimicrobial Therapy

Experience with antimicrobial treatment of *C. upsaliensis* infection is limited. In vitro testing reveals that the organism is typically sensitive to aminoglycosides, cephalosporins, tetracycline, and nalidixic acid. It is also usually sensitive to erythromycin, but resistant strains have been described (39, 70). *C. upsaliensis* strains are generally resistant to vancomycin, methicillin, piperacillin, and chloramphenicol (70).

Patients with *C. upsaliensis* bacteremia have been successfully treated with erythromycin (39, 70). Chusid et al. (21) documented the eradication of *C. upsaliensis* from blood cultures in a patient after 5 days of treatment with cefotaxime. In a study in Belgium (41), 11 patients with diarrhea and *C. upsaliensis* in stools received erythromycin and 2 received amoxicillin. The diarrheal symptoms disappeared and the organism was eradicated in all 13 patients receiving therapy. However, there have been no controlled trials of antibiotic treatment for *C. upsaliensis*-associated diarrhea. Therefore, the place of antibiotic therapy for use in the treatment of infection by *C. upsaliensis* has yet to be defined.

Although clinical studies provide evidence suggesting the importance of *C. upsaliensis* as a human enteropathogen, it has to be emphasized that each of the studies to date is uncontrolled. There is therefore a pressing need for controlled studies comparing *C. upsaliensis* isolation rates in patients with diarrhea with the rates in age-matched, asymptomatic controls. Experimental challenge of human volunteers and a variety of animal models are also required to fulfill each of Koch's postulates and thereby confirm the enterovirulence of *C. upsaliensis*.

VIRULENCE PROPERTIES

Toxin production, adhesion to mucosal surfaces, and invasion and replication of organisms within epithelial cells are etiopathogenetic features of many bacterial infections (30).

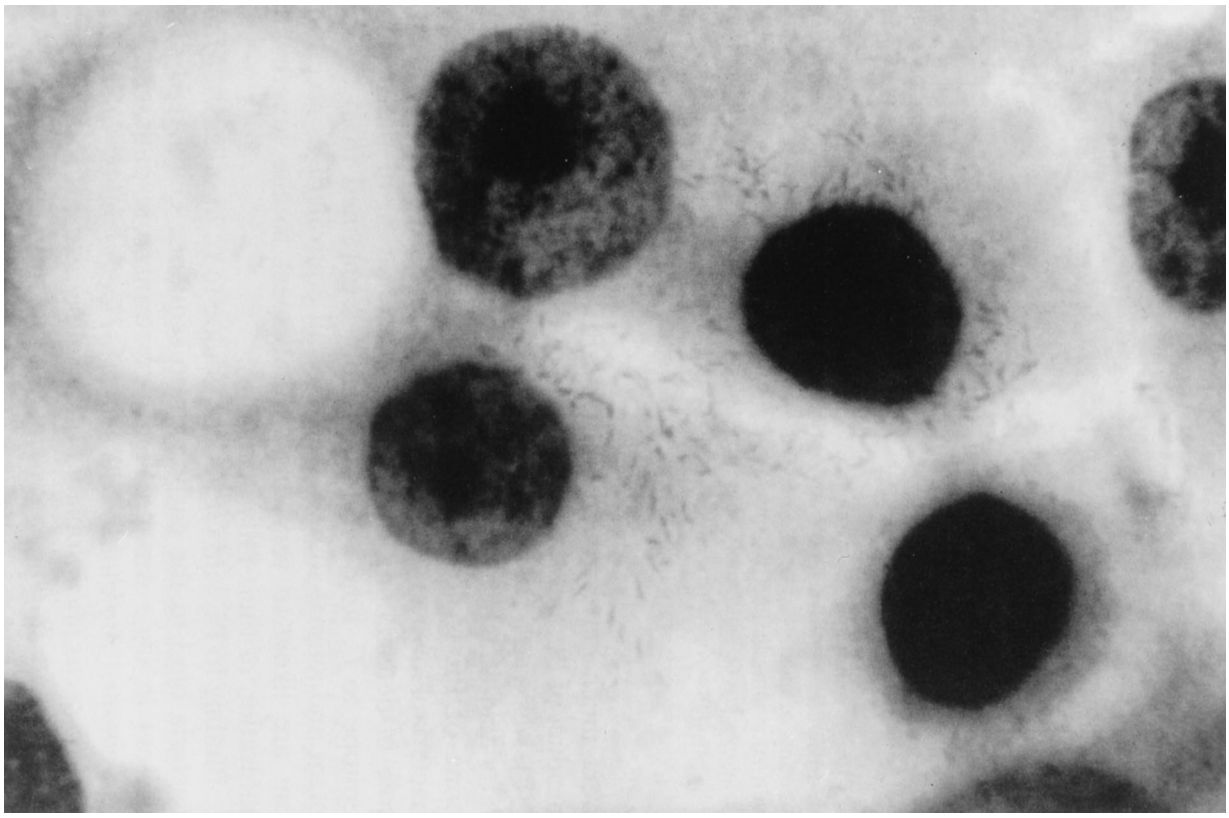

FIG. 2. Electron micrograph (magnification, ca. ×800) demonstrating HEp-2 cells with adherent *C. upsaliensis* organisms. Reprinted from reference 85 with permission of the publisher.

Colonization and infection by bacteria are initially dependent on the interaction of the bacteria with host surfaces. Bacterial adherence is therefore necessary before an organism can cause disease. Toxin production is used by many bacteria to cause damage to host cells, although the precise role of many toxins remains to be clarified. Invasion by organisms of host epithelial cells gives access to a rich nutrient environment and is also used to avoid host immune responses. Microbial pathogenicity is increasingly recognized as multifactorial, with many organisms using multiple complementary mechanisms to produce human disease (30).

The mechanisms by which enteric campylobacters cause human disease have not been clarified. In fact, of all the *Campylobacter* species, only *C. jejuni* has been studied in detail. Possible virulence factors in *C. jejuni* include flagellin (1), enterotoxin production (76), cytolethal distending toxin production (49), microbial adherence (57), and invasion of intestinal epithelial cells (26). In addition, some *C. jejuni* isolates demonstrate cytotoxic activity on HeLa and Chinese hamster ovary (CHO) cells (43). The relative importance, if any, of each of these mechanisms to the production of disease in humans remains to be determined. As illustrated in a recent comprehensive review of the field (51), the application of genetic techniques to the study of *Campylobacter* virulence mechanisms is required to more precisely determine the method by which these organisms cause disease. For instance, the generation of isogenic mutants lacking the gene(s) for putative virulence properties is the definitive way to elucidate the relative importance of individual virulence gene products.

The systematic study of virulence mechanisms in *C. upsaliensis* is in its infancy. Thus far, information on the pathophysiology of infection with this organism comes from only a handful of preliminary reports, which are considered in more detail below.

Motility

As with other *Campylobacter* species, *C. upsaliensis* is motile and has either a single flagellum or bipolar flagella. It is known that flagellar antigens show strong cross-reactivity among the *Campylobacter* species. A study in Toronto has shown that there is a flagellar antigen common to *C. upsaliensis* and other campylobacters of documented pathogenicity in humans (e.g., *C. jejuni* and *C. coli* (63). Therefore, the putative role of flagellin in the virulence of other *Campylobacter* species may also apply to *C. upsaliensis*. However, there have been no published investigations specifically evaluating the potential role of *C. upsaliensis* flagella as a virulence factor for the organism.

Adherence

Megraud et al. (62) reported that 16 CNW strains adhered to an endothelial cell monolayer in a similar fashion to other *Campylobacter* spp. An average of only 30% of the endothelial cells were infected in this study, and most of these infected cells had only a few adherent bacteria. This low level of adhesion may simply reflect the choice of cell line used in the study.

In the first in-depth study of the virulence of *C. upsaliensis*, Sylvester et al. (85) recently showed that *C. upsaliensis* binds to CHO and HEp-2 cells in tissue culture (Fig. 2). The manner in which *C. upsaliensis* bound to epithelial cells in vitro was comparable to the diffuse adherence pattern of *C. jejuni*. The authors also demonstrated the binding of *C. upsaliensis* to

human small intestinal mucin and characterized the adherence of *C. upsaliensis* to intestinal lipids. In a thin-layer chromatography overlay binding assay, *C. upsaliensis* bound to phosphatidylethanolamine, phosphatidylserine, and gangliotetraosylceramide. Affinity was highest for phosphatidylethanolamine, and this lipid was detected in lipid extracts from three different cell lines (CHO, HEL, and HEp-2). Further work is needed to more exactly determine the nature and relevance in vivo of mucin and membrane lipid binding by this organism.

Invasion

To date, there have been no reports on the potential role of bacterial internalization into the cytosol of host epithelial cells as a virulence mechanism for *C. upsaliensis*.

Toxin Production

Figura et al. (29) examined a number of atypical campylobacters for the production of cytotonic, cytotoxic, and cytolethal distending toxins. Each of the two *C. upsaliensis* strains tested in this study produced a cytolethal distending toxin. The presence or absence of cytotoxic and cytotonic toxins in these *C. upsaliensis* strains was not discussed. More recently, Pickett et al. (73) confirmed the likely presence of a *cdtB* homolog in the *C. upsaliensis* type strain. However, the putative *C. upsaliensis cdt* gene(s) has yet to be cloned.

Regardless of the identification of putative virulence factors for *C. upsaliensis*, proof of its role as a human enteropathogen will require the fulfillment of each of Koch's postulates. Of these four postulates, only the second (i.e., the isolation of the organism from diseased subjects) has been demonstrated for *C. upsaliensis*. Therefore, there is a pressing need for controlled studies comparing isolation rates in symptomatic and asymptomatic controls and for animal (and human) challenge studies with subsequent reisolation of *C. upsaliensis* before this organism receives unequivocal recognition as a cause of human disease.

MOLECULAR BIOLOGY

Compared with many other gram-negative organisms such as *E. coli* and *Salmonella* spp., progress in *Campylobacter* molecular biology has been slow. For instance, natural transformation has only recently been described for campylobacters (94, 95). Moreover, the generation of *E. coli*-to-*Campylobacter* shuttle vectors (54) and suicide vectors (53) has occurred only relatively recently. In addition, a number of workers encountered difficulties in isolating and cloning genes in enteric campylobacters (87). These problems included failure of *Campylobacter* gene expression and instability of genes cloned in *E. coli* (16, 60). A number of possible reasons for these difficulties have been postulated, including differences in codon usage, methylation, and accessory gene requirements between *Campylobacter* species and *E. coli* (87). Many of these initial difficulties have now been overcome, and the genetic characterization of the campylobacters is proceeding more rapidly.

Until recently, the molecular biology of *C. upsaliensis* was largely unexplored. A number of investigators (23, 82) commented on the relative frequency of plasmids isolated from strains of *C. upsaliensis* (60 to 93%), which is considerably more frequent than the plasmid carriage of other campylobacters such as *C. jejuni*. Plasmid carriage appears to be more common among *C. upsaliensis* strains isolated from humans (82). However, there has been no systematic structural or functional study of these extrachromosomal genetic elements. Other isolated observations on the genetics of this organism include the presence of methylated chromosomal DNA (25) and internal transcribed spacers in 23S rRNA genes (27); both these features are found in other *Campylobacter* species also.

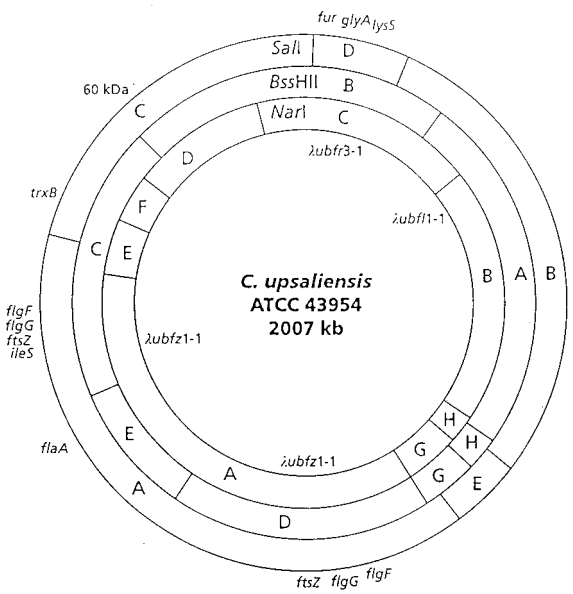

FIG. 3. Physical genetic map of *C. upsaliensis* ATCC43954 generated with *Sal*I, *Nar*I, and *Bss*HII. Reprinted (with a slight modification) from reference 9 with permission of the publisher.

As an initial step toward understanding the molecular events involved in the pathogenesis of *C. upsaliensis* infection, we constructed a physical-genetic map of the chromosome of this organism (9) (Fig. 3). The genome of the *C. upsaliensis* type strain, ATCC 43954, is over 2 Mb and, therefore, considerably larger than the genomes of other enteric campylobacters such as *C. jejuni* (1.8 Mb) (52) and *C. coli* (1.7 Mb) (97). Additional studies indicate a range of genomic sizes (1.74 to 2.09 Mb) among clinical isolates of *C. upsaliensis* (11). Since the *C. upsaliensis* type strain appears to harbor an extensive duplication, it is possible that the increased size of some *C. upsaliensis* isolates is due to the presence of chromosomal duplications in these strains.

At a macrorestriction level, *C. upsaliensis* demonstrates considerable genomic heterogeneity (11) (Fig. 1) reminiscent of the *H. pylori* genome (87). This finding is particularly intriguing in light of the relative evolutionary closeness of these two organisms and, conversely, the apparent dissimilarity of their respective ecological niches. However, preliminary evidence (12) does not suggest the presence of interstrain chromosomal rearrangement of genes in *C. upsaliensis*, which accounts for the remarkable variation observed among individual strains of *Helicobacter pylori* (48).

To identify de novo virulence-related genes in *C. upsaliensis*, we recently cloned and sequenced the iron uptake regulatory (*fur*) gene for this species (12). Fur acts as a transcriptional regulator repressing the expression of genes involved in iron homeostasis (42), the acid tolerance response (31), and general metabolism (90), as well as virulence genes (15, 67) and genes involved in protection of the organism from oxidative stress (89). Interestingly, the arrangement of genes downstream of *C. upsaliensis fur* is identical to that observed in *C. jejuni* (12, 18–20, 96). In fact, there is 100% identity at the nucleotide level across the regions spanning the junctions between *fur* and the two downstream open reading frames (Fig. 4). PCR exper-

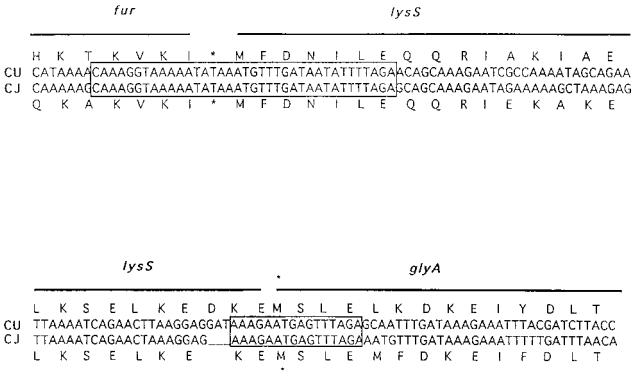

FIG. 4. Comparison of the gene arrangement downstream of *fur* in *C. upsaliensis* and *C. jejuni*. The upper diagram depicts the junction of the *fur* and *lysS* genes, and the lower diagram depicts the junction of the *lysS* and *glyA* genes. The *C. upsaliensis* (CU) DNA sequence is aligned above the respective *C. jejuni* (CJ) sequence. The deduced amino acid sequence for each organism is indicated above (*C. upsaliensis*) and below (*C. jejuni*) the respective nucleotide sequences. Asterisks indicate termination codons. Reprinted from reference 12 with permission of the publisher.

iments indicate that the *fur-lysS-glyA* arrangement of genes is highly conserved among the three human enteric campylobacters *C. jejuni*, *C. upsaliensis*, and *C. coli*. Northern analysis indicates the expression of polycistronic *fur* transcripts that probably encode Fur and LysS in both *C. upsaliensis* and *C. jejuni* (12, 20). The exact reason for the conservation of this close arrangement of apparently unrelated genes is unknown. However, it is noteworthy that a number of amino acyl-tRNA synthetases, including LysS, catalyze the synthesis of polyadenylated nucleotides whose levels are elevated when cells are exposed to heat or oxidative stress (13). It is conceivable that clustering of amino acyl-tRNA synthetase and *fur* genes in diverse bacterial species has a functional relevance and may be related to their complementary roles in cellular protection from oxidative stress.

CONCLUSIONS

Failure to identify *C. upsaliensis* in the setting of many clinical microbiology laboratories is undoubtedly related to the sensitivity of this organism to the antibiotics routinely used in *Campylobacter* selective media. As a result, in the realm of clinical microbiology *C. upsaliensis* remains relatively obscure. Because it has received such scant attention, our understanding of this enteric campylobacter has lagged far behind that of related pathogens such as *C. jejuni*.

A considerable body of mainly epidemiological evidence now indicates the potential importance of *C. upsaliensis* as a cause of human enteric infection. With the development of improved isolation techniques, we anticipate renewed interest in this organism by those involved in the study of pathogenic bacteria. Future studies aimed at elucidation of the precise role of this organism in human disease and identification of its pathogenic mechanisms ultimately will allow the development of effective strategies aimed at treatment and prevention of *C. upsaliensis*-related infections.

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