JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1989, p. 668–670
0095-1137/89/040668-03$02.00/0

Vol. 27, No. 4

Direct Isolation of Atypical Thermophilic *Campylobacter* Species from Human Feces on Selective Agar Medium

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Received 21 October 1988/Accepted 19 December 1988

Campylobacter upsaliensis **is the name which has been proposed for a new group of thermophilic campylobacter strains which differ from *C. jejuni* and *C. coli* in having a negative or weak catalase reaction. Primary isolation of these strains from human feces has been achieved only by use of filtration techniques. We report here direct isolation of strains corresponding to *C. upsaliensis* from stools of six children. The strains were isolated on a newly described campylobacter-selective medium. The strains were oxidase positive, hippurate negative, nitrate positive, negative for H_2S in triple sugar iron, and susceptible to cephalothin (30-μg disk) and nalidixic acid (30-μg disk), and they grew at 37 and 43°C, but not at 25°C. The selective medium used was a blood-free, charcoal-based medium consisting of Columbia agar base, activated charcoal, cefoperazone (32 μg/ml), vancomycin (20 μg/ml), and cycloheximide (100 μg/ml). The medium supported the growth of the weakly reacting or catalase-negative strains, with colony counts equivalent to those obtained on antibiotic-free horse blood agar. These strains could not be isolated directly from stool on Skirrow medium, and colony counts confirmed that this medium could not support a low inoculum of these organisms. The clinical significance of these strains is unknown. We conclude that *C. upsaliensis* can be isolated directly from stool by using a selective medium, without the need for filtration.**

In 1983, Sandstedt and colleagues (9) reported on the isolation of unusual thermotolerant *Campylobacter* species from feces of diarrheic and healthy dogs. These strains differed from *Campylobacter jejuni* and *Campylobacter coli* by having a weak or negative catalase reaction and were referred to as CNW (catalase negative or weakly reacting). The CNW thermotolerant campylobacters were shown to represent a distinct species on the basis of DNA-DNA hybridization studies.

Steele et al. (12), using a filtration technique, subsequently recovered CNW-like strains of *Campylobacter* spp. from young children admitted with gastroenteritis to hospitals in Central Australia. Further characterization by disk susceptibility tests revealed that these strains differed from the *C. jejuni* and *C. coli* groups in being susceptible to cephalothin (zone diameter, 20 to 34 mm), colistin (zone diameter, 18 to 22 mm), and nalidixic acid (zone diameter, 18 to 28 mm). None of these susceptible strains formed visible colonies after 2 days of incubation at 37°C on Skirrow-type selective medium (10) (blood agar plates containing vancomycin [10 μg/ml], trimethoprim [5 μg/ml], and polymyxin B [2,500 IU/liter] or colistin [10,000 U/liter]). In addition, no growth occurred on selective media containing cephalothin. DNA-DNA hybridization studies showed a high degree of homology with the CNW strains described earlier by Sandstedt et al. (9), suggesting relatedness.

Five phenotypically similar strains were isolated by Megraud and Bonnet (6) from stools of diarrheic children in Bordeaux, France. Again, a filtration technique and blood agar were used for primary isolation. The protein profiles of these organisms by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were similar to those of Sandstedt.

Sandstedt and Ursing (Abstr. 14th Int. Congr. Microbiol. 1986, P.B 8-17.) have recently proposed the name *Campylobacter upsaliensis* for these strains.

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Since 1986, we have routinely employed a charcoal-based selective medium (CSM) (5), in addition to a Skirrow-like medium (SKM), to isolate thermophilic campylobacters from stool. We report here the direct isolation on CSM of CNW-like thermophilic *Campylobacter* spp. from stools of six children.

MATERIALS AND METHODS

Media. The two campylobacter-selective media used routinely in our laboratory are (i) CSM, containing the selective agents vancomycin (20 μg/ml), cycloheximide (100 μg/ml), and cefoperazone (32 μg/ml), and (ii) SKM, containing trimethoprim lactate (5 μg/ml), polymyxin B (0.25 μg/ml), and vancomycin (10 μg/ml). The specific compositions of these media have been described previously (5).

Isolation procedure. The Hospital for Sick Children is a 585-bed tertiary care pediatric hospital serving the City of Toronto, Ontario, Canada, and surrounding centers, with referrals mainly from southwestern Ontario, but from other Canadian centers as well.

All stools received in the microbiology lab are routinely cultured by standard methods for *Salmonella* spp., *Shigella* spp., and *Yersinia enterocolitica*, as well as for thermophilic *Campylobacter* spp., on CSM and SKM. The campylobacter-selective media are incubated at 43°C in anaerobic jars under reduced oxygen tension (approximately 7%) (3). In our laboratory this is achieved by evacuating two-thirds of the air from the jars without a catalyst and replacing it with a mixture of carbon dioxide and hydrogen gas to a final estimated concentration of 7% oxygen, 10% carbon dioxide, 25% nitrogen, and 58% hydrogen (5). The plates are examined for *Campylobacter* species after 1 and 2 days of incubation.

Identification of *Campylobacter* species and strains. Suspect colonies on selective media are examined microscopically on wet mounts for typical gram-negative curved bacteria with darting motility. Species are identified by a panel of bio-

TABLE 1. Biochemical characterization of enteric *Campylobacter* spp.

Species	Biochemical characteristics										
	Catalase	Oxidase	Growth at (°C):			Hippurate	Nitrate	Production of H_2S on:		Nalidixic acid[a]	Cephalothin[a]
			25	37	43			Skirrow	TSI[b]		
C. jejuni	+	+	−	+	+	+	+	−/+	−	S	R
C. coli	+	+	−	+	+	−	+	−	−	S	R
C. fetus	+	+	+	+	−	−	+	−	−	R	S
C. laridis	+	+	−	+	+	−	+	+	−	R	R
C. hyointestinalis	+	+	+/−	+	+/−	−	+	+	+	R	S
CNW-like	− or weakly +	+	−	+	+	−	+	−	−	S	S

[a] 30 μg. R, Resistant; S, susceptible.
[b] Triple sugar iron.

chemical tests (4, 8, 11) routinely used in our laboratory (Table 1).

Ability of media to support low inocula. The ability of CSM, blood agar, and SKM to support the growth of small inocula of CNW-like thermophilic *Campylobacter* spp. was determined by examining viable counts (7) of suspensions. Media were incubated for 48 h at both 37 and 43°C.

RESULTS

During the period from January 1986 to April 1987, thermophilic campylobacters were isolated from the stools of 86 children (Fig. 1). CNW-like strains isolated from the stools of six children represented 7% of all isolates. These atypical strains were all isolated during a 4-week period from 14 October to 11 November 1986 and represented 66% of all *Campylobacter* spp. isolates during this 28-day interval. The CNW isolates were initially detected on the CSM plates but were not apparent on the SKM plates. Phenotypic characterization (Table 1) showed these strains were similar to the CNW strains isolated by Sandstedt et al. (9), Steele et al. (12), and Megraud and Bonnet (6).

Viable counts (median of at least four determinations) of these atypical campylobacters were equivalent on blood agar and CSM (Table 2). Both media were markedly superior to SKM. Skirrow medium was unable to support the growth of low inocula of this organism ($<10^7$ organisms per ml).

The six children with fecal isolates of CNW *Campylobacter* spp. were negative for other bacterial enteric pathogens. They comprised three males and three females with an average age of 20 months (range, 3.5 to 36 months). Five were inpatients of the Hospital for Sick Children at the time their isolates were obtained.

Three of the children had signs and symptoms compatible with gastroenteritis (loose watery stool, vomiting, and anorexia) at the time the positive isolate was obtained. The illness was self-limiting in two of these children; child 3 received a 10-day course of erythromycin following a 3-week protracted illness with five to six watery stools per day.

Two patients, both asymptomatic, had occupied the same hospital room for over 1 week at the time their positive isolates were obtained. Contact with another child whose stool cultures were positive for *Salmonella* species had prompted the stool examination in these children. Stool cultures were done for child 6, who had no gastrointestinal symptomatology, as part of a work-up for unidentified fever.

DISCUSSION

Sandstedt et al. (9) were the first to isolate CNW *Campylobacter* spp. from the stools of diarrheic and healthy dogs in Sweden. These strains represented 64% of their isolates. These organisms formed a genetically separate group, with G+C content more than 3% higher than that of any other thermotolerant strain (13). DNA relatedness to *C. jejuni* and *C. coli* was about 40%.

Subsequently, Steele et al. (12) recovered eight CNW isolates from diarrheic stools of Australian children and one

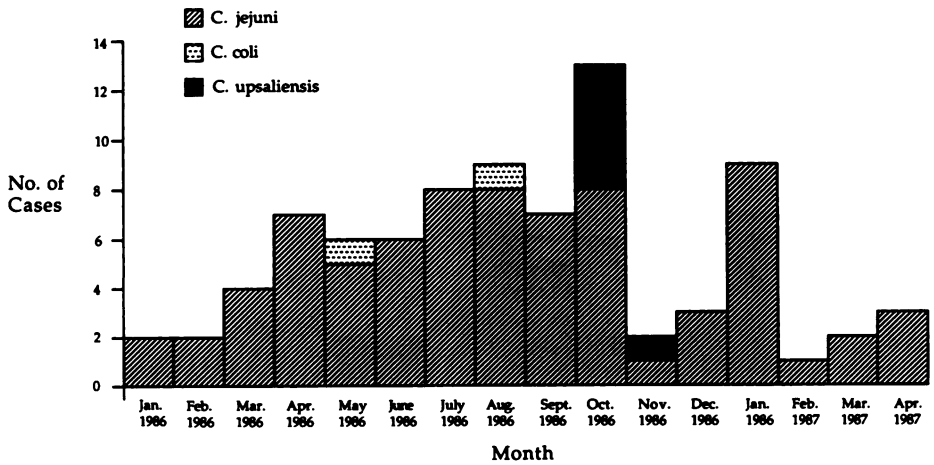

FIG. 1. Relative frequency of isolation of thermophilic campylobacters at the Hospital for Sick Children, Toronto, Canada, January 1986 to April 1987.

TABLE 2. Effects of media and temperature on the viable count of CNW-like strains of *Campylobacter* spp.

Strain	Viable count[a] at:				
	37°C		43°C		37 or 43°C on SKM
	BA	CSM	BA	CSM	
6800	2×10^7	2×10^5	2×10^8	5×10^7	$<1 \times 10$
6648	1×10^7	7×10^6	3×10^6	2×10^7	$<1 \times 10$
6619	2×10^7	6×10^4	1×10^8	8×10^6	$<1 \times 10$

[a] Median count, more than four determinations. BA, Blood agar.

from an adult, representing 11% of their *Campylobacter* isolates. DNA hybridization studies revealed >70% binding with homologous strains but less than 15% binding with a variety of other *Campylobacter* species. CNW isolates represented 13% of the *Campylobacter* isolates of Megraud and Bonnet (6).

Our six isolates of CNW *Campylobacter* spp. are phenotypically similar to the strains described above and represent human isolates of the new species *C. upsaliensis*.

All of the previous investigators (6, 9, 12) used a filtration technique and nonselective blood agar for the initial isolation of their strains. Following primary isolation, the canine isolates of Sandstedt (9) grew well on SKM at 42°C, whereas none of the human isolates of Steel (12) grew on SKM after 48 h at 37°C, although two strains formed small colonies after 5 days of incubation. The human isolates of Megraud (6) grew on SKM. We have shown by viable-count techniques that an SKM used in our laboratory is unable to support the growth of low inocula of these organisms. An inoculum effect may be one explanation for the difference in success of isolation on SKM by other investigators (6, 9, 12).

The fact that CSM supports the growth of low inocula of the CNW strains suggests that this medium would be useful for screening stool for this organism. Filtration techniques have a low sensitivity (2), with an upper limit of 10^6 CFU/g of stool (6), and are more cumbersome than selective agar media. The presence of cefoperazone, rather than colistin, cephalothin, or polymyxin, probably explains our success in isolating these organisms. The strains isolated by Steele (12) were susceptible to these other antibiotics.

We have previously reported on the use of CSM for the isolation of *Campylobacter* spp. from stool (5). CSM was found to be more selective than SKM, with a higher isolation rate of *Campylobacter* species than the latter. It was concluded that the use of both media together would be an optimal combination for the isolation of thermophilic campylobacters from stool. Indeed, had Skirrow medium alone been used for the isolation of *Campylobacter* spp., as is customary in many hospital laboratories, none of our isolates of CNW strains would have been detected.

The potential role of *C. upsaliensis* as a human enteric pathogen requires further evaluation. The identification of an effective primary isolation medium should help make these studies possible. The fact that all the isolates of CNW-like organisms appeared over a 4-week period may be significant. Seasonal variation rates in the isolation of other enteric *Campylobacter* spp. have previously been noted (1). Isolation from hospitalized patients, including two asymptomatic children, raises the issue of possible nosocomial spread. Symptoms consistent with gastroenteritis did occur in three of our patients, although cause and effect could not be confirmed. Steele et al. (12) and Megraud and Bonnet (6) also isolated these organisms from children with symptoms of gastroenteritis. Isolation of these organisms from asymptomatic children also suggests the potential for asymptomatic carriage. Similarly, this organism has been isolated from both healthy and diarrheic dogs (9). Patton et al. (C. M. Patton, N. Shaffer, P. Edmonds, T. Bourrett, M. A. Lambert, C. Baker, A. M. Perlman, and D. J. Brenner, Abstr. 4th Int. Workshop on *Campylobacter* Infect., abstr. no. 120, 1987) describe another 11 clinical isolates of this species, including 8 isolates from blood. These observations suggest that *C. upsaliensis* may be associated with a variety of clinical illnesses in the normal and compromised host.

We conclude that on the basis of phenotypic characteristics, our six isolates of CNW-like *Campylobacter* spp. from stool represent strains of *C. upsaliensis* recently described by Sandstedt and Ursing (Abstr. 14th Int. Congr. Microbiol. 1986). The clinical significance of these organisms remains to be investigated. The fact that CSM permits primary isolation of these organisms from feces will allow for subsequent studies to determine their clinical and epidemiological significance.

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