Selective medium for thermophilic campylobacters including *Campylobacter upsaliensis*

S T Aspinall, D R A Wareing, P G Hayward, D N Hutchinson

Abstract

Aims—To develop a selective supplement for the recovery of thermophilic *Campylobacter* spp, including *Campylobacter upsaliensis* from faeces, using campylobacter blood free selective agar base as the growth medium.

Methods—Minimum inhibitory concentrations (MICs) of cefoperazone and of teicoplanin were determined for 51 strains of *C upsaliensis*, 159 strains of other thermophilic *Campylobacter* spp, and for 66 *Enterobacteriaceae* (cefoperazone only). From these results a medium using campylobacter blood free selective agar base incorporating cefoperazone (8 mg/l), amphotericin (10 mg/l), and teicoplanin (4 mg/l)—CAT medium—was formulated and compared with the commercially available campylobacter blood free selective medium (modified CCDA) for isolation of *Campylobacter* spp from 7000 human faecal specimens. The two media were also compared for the recovery of *C upsaliensis* from 45 spiked human faeces.

Results—Isolation rates of *Campylobacter* spp other than *C upsaliensis* were similar for both media, but the CAT medium alone recovered four of the five strains of *C upsaliensis* from the faecal samples examined. From the spiked faeces specimens, recovery of *C upsaliensis* was increased by between 35% and over 200-fold on the CAT medium compared with the modified CCDA.

Conclusions—CAT selective agar was a suitable alternative medium to modified CCDA for the growth of thermophilic *Campylobacter* spp, including *C upsaliensis* from faeces.

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In 1983 a group of thermophilic, catalase negative or weakly positive (CNW) *Campylobacter* spp was isolated from dogs.¹ DNA hybridisation studies showed that this group belonged to a new species and the name *Campylobacter upsaliensis* was proposed.² More recently, several reports have suggested that *C upsaliensis* is a potential human pathogen associated with enteritis, bacteraemia, and opportunist infections in immunocompromised individuals,³⁻⁸ with studies suggesting that 3-13% of all campylobacter diarrhoeal disease is associated with

this organism.^{9,10} Dogs could be one possible source of infection.^{11,12}

To elucidate the aetiology of such infections, suitable culture media are required to determine the prevalence of such organisms. Fewer than 20% of strains, however, have been reported to grow on the currently used campylobacter selective media and a filtration method using a non-selective medium is the technique advocated for its isolation.^{8,9}

Methods

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS (MIC)

Antibiotics

Cefoperazone (Unipath Ltd, Basingstoke, England) and teicoplanin (Merrell Dow Pharmaceuticals Ltd, Uxbridge, England) were supplied as a freeze dried supplement and a powder, respectively.

Micro-organisms

The following organisms were tested: 51 strains of *C upsaliensis*, 140 strains of *C jejuni* subspecies *jejuni*, and 19 strains of *C coli* isolated from dog, cat, and human faeces using the membrane filtration culture technique,^{13,14} followed by incubation for 48 hours at 37°C in a microaerobic atmosphere.

Strains of *Enterobacteriaceae* (n = 66) obtained from human faeces using MacConkey agar (Unipath Ltd) were also examined.

Isolates of *Campylobacter* spp were identified by accepted methods,¹⁵ while *Enterobacteriaceae* were identified using API 20E (API bioMerieux, Basingstoke, England).

Method

Doubling dilutions of cefoperazone from 2 mg/l to 128 mg/l and teicoplanin from 8 mg/l to 64 mg/l were prepared in campylobacter blood free selective agar base (Unipath Ltd) containing an additional 0.8% (w/v) bacteriological agar No 1 (Unipath Ltd), resulting in a final agar concentration of 2%. Isolates for testing were cultured on Columbia blood agar containing 5% (v/v) whole horse blood for 48 hours at 37°C in a microaerobic atmosphere. The bacteria were then suspended in 0.1% bacteriological peptone and adjusted to an optical density of 0.2 absorbance units at 465 nm using a BioRad 3550 microplate reader (BioRad Laboratories Ltd, Hemel Hempstead, England). This absorbance is equivalent to 10⁹ colony forming units/ml, as determined by the method of

Public Health Laboratory, Royal Preston Hospital, PO Box 202, Preston, Lancs PR2 4HG
S T Aspinall
D R A Wareing
P G Hayward
D N Hutchinson

Correspondence to: S T Aspinall, Department of Microbiology, Royal Albert Edward Infirmary, Wigan Lane, Wigan WN1 2NN.

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Miles and Misra.¹⁶ The suspensions of organisms were then diluted 1 in 1000 and inoculated on to the antibiotic containing media together with an equivalent antibiotic free medium, using a Denley multipoint inoculator (Denley Instruments Ltd, Billingshurst, England) with a 21 pin head. All plates were incubated at 37°C for 48 hours in a microaerobic atmosphere after which the MIC was defined as the lowest concentration of antibiotic at which growth was completely inhibited. The *Campylobacter* spp were tested against both cefoperazone and teicoplanin, while the *Enterobacteriaceae* were only tested against cefoperazone.

FORMULATION OF A NEW SELECTIVE MEDIUM

Campylobacter blood free selective base was prepared using 8 mg/l of cefoperazone, 10 mg/l of amphotericin (Bristol-Myers Squibb Pharmaceuticals Ltd, Hounslow, England), and 4 mg/l of teicoplanin (CAT medium). Strains of *Campylobacter* spp included: *C. upsaliensis* (n = 40), *C. jejuni* subspecies *jejuni* (n = 40), *C. coli* (n = 10), *C. lari* (n = 10), *C. cryaerophila* (n = 3) and *C. hyointestinalis* (n = 3) were inoculated on to both the CAT medium and an equivalent antibiotic free medium, spread for isolated colonies, and incubated at 37°C for 48 hours in a microaerobic atmosphere. After incubation all cultures were examined for typical growth and colonial morphology.

COMPARISON OF CAT AND MODIFIED CCDA-PRESTON MEDIUM FOR ISOLATION OF *CAMPYLOBACTER* SPP FROM HUMAN FAECES

From human faeces

Modified CCDA-Preston medium (*campylobacter* blood free selective agar base, containing 32 mg/l of cefoperazone and 10 mg/l of amphotericin (Unipath Ltd)) was compared with CAT medium for the isolation of *Campylobacter* spp from 7000 human faeces submitted to Preston Public Health Laboratory for routine culture.

Using a 1 in 10 dilution of faeces (0.5 g in 5 ml) in 0.1% (w/v) bacteriological peptone, the media were inoculated using a sterile swab, spread for discrete colonies, and incubated at 37°C for 48 hours in a microaerobic atmosphere. Any suspect colonies were identified as described before.¹⁵

Growth of all isolates of *Campylobacter* spp (and for the first 1000 specimens, all isolates other than *Campylobacter* spp) were graded: scanty (growth only in the primary inoculum); moderate (growth in the primary and

secondary streaks); and heavy (growth in all streaks).

From spiked human faeces

Strains of *C. upsaliensis* (n = 45) were inoculated into 45 different faecal samples at final concentrations of (a) 10⁵–10⁶ cfu/g and (b) 10⁷–10⁸ cfu/g. Modified CCDA and CAT media were inoculated and incubated as above and recovery of the isolates recorded.

Results

MIC DETERMINATIONS

Teicoplanin

All *Campylobacter* spp had MICs to teicoplanin of >64 mg/l on the *campylobacter* blood free selective base.

Cefoperazone

Most *C. upsaliensis* strains (47 out of 51) had MICs of ≥16 mg/l to cefoperazone (table), whilst all strains of *C. jejuni* and *C. coli* had MICs of >128 mg/l. All 66 strains of *Enterobacteriaceae* tested has MICs of ≤8 mg/l.

GROWTH OF *CAMPYLOBACTER* SPP ON CAT MEDIUM

All isolates of *Campylobacter* spp tested yielded equivalent growth on both the CAT selective medium and the antibiotic free medium.

COMPARISON OF CAT WITH MODIFIED CCDA

From human faeces

Selectivity (1000 faeces) The same amount of growth of contaminants was found on both media in 91% of cultures; on CAT medium a small, moderate, or heavy increase in isolates other than *Campylobacter* spp was seen in 5.5%, 2%, and 0.5% of cultures, respectively. Conversely, 1% of cultures gave a moderate increase in contaminants on the modified CCDA compared with CAT medium.

Isolation of *Campylobacter* spp (7000 faeces)

From the 7000 faecal specimens, 229 isolates of *Campylobacter* spp were recovered. These comprised 209 strains of *C. jejuni* subspecies *jejuni*, 13 strains of *C. coli*, two strains of *C. lari* and five strains of *C. upsaliensis*. Both CAT and modified CCDA media yielded similar growths of *Campylobacter* spp (other than *C. upsaliensis*) with the CAT medium recovering 223 of the 224 isolates and the modified CCDA recovering 221. Four of the strains of *C. upsaliensis* isolates, however, were only recovered on the CAT medium and would not grow on the modified CCDA even after subculture.

From spiked human faeces

From the faecal specimens spiked with *C. upsaliensis* at a concentration of 10⁷–10⁸ cfu/g, the organism was recovered using CAT medium on 42 occasions, while it was isolated from 31 samples using modified CCDA. At a concentration of 10⁵–10⁶ cfu/g *C. upsaliensis* was isolated from CAT and modified CCDA on 37 and 11 occasions, respectively.

Minimum inhibitory concentrations of *C. upsaliensis*, *C. jejuni* subspecies *jejuni*, *C. coli* and *Enterobacteriaceae* to cefoperazone

	MIC of cefoperazone (mg/l)						
	≤2	4	8	16	32	64	>128
<i>C. upsaliensis</i> (51)	3	0	1	3	20	12	12
<i>C. jejuni</i> spp <i>jejuni</i> (140)							140
<i>C. coli</i> (19)							19
<i>Enterobacteriaceae</i> (66)	53	1	12				

Discussion

Campylobacter upsaliensis has been implicated as a potential human pathogen by several workers,³⁻⁸ but its isolation from faeces using selective media has posed considerable problems due to the wide range of antibiotic susceptibilities observed for these organisms.⁸ The recommended method for isolation of *C upsaliensis* involves a membrane filtration culture technique but this not only incurs the additional cost of media and filters, and the need to leave the inoculated filter on the agar for 45 minutes before removal and incubation, but also results in a lack of recovery of the organism from faeces containing less than 10^5 cfu/g.¹⁰ Therefore, we formulated a new selective supplement to be added to the commercially available campylobacter blood free selective agar base containing charcoal, which would permit the isolation of thermophilic *Campylobacter* spp and improve the recovery of *C upsaliensis* from faeces. The reduced concentration of cefoperazone (8 mg/l rather than 32 mg/l) inhibited most *Enterobacteriaceae*, but not faecal streptococci; therefore teicoplanin (4 mg/l) was included. Amphotericin was also included as an anti-fungal agent at a concentration of 10 mg/l, as used in the modified CCDA. At these concentrations, the newly formulated medium (CAT) did not affect the growth or colonial morphology of any of the *Campylobacter* spp tested, yet allowed the growth of 47 of the 51 isolates of *C upsaliensis* tested.

In 1990 Goossens *et al*⁸ reported that 48 of 91 isolates of *C upsaliensis* examined had MICs to cefoperazone of ≤ 6.25 mg/l when tested in Müller-Hinton broth. We found, however, that when cultured on a charcoal based solid agar medium only four of 51 isolates of *C upsaliensis* had MICs of ≤ 8 mg/l. One of the most likely explanations for this is the ability of charcoal to bind antibiotics with the result that the antibiotic effect is reduced.

When CAT medium was compared with modified CCDA for the isolation of thermophilic campylobacters from 7000 human faeces, isolation rates of *Campylobacter* spp other than *C upsaliensis* were similar. Five (2.2%) of the 229 *Campylobacter* spp isolated, however, were *C upsaliensis* and of these, four were isolated only on the CAT medium. The isolation rate for *C upsaliensis* in this study was similar to that reported before from this laboratory,¹⁷ but some workers have reported that up to 13% of campylobacter diarrhoeal disease is associated with this organism.¹⁰

To investigate the recovery of *C upsaliensis* using CAT medium compared with modified CCDA, 45 randomly selected campylobacter culture negative faecal samples were spiked with different strains of the organism at concentrations between 10^5 and 10^6 cfu/g and also between 10^7 and 10^8 cfu/g. At the lower concentration 82% (37) of the strains were recovered on the CAT medium; only 24% (11) were recovered on modified CCDA. This particular concentration was chosen as it

is approaching the level below which the membrane filtration culture technique has been reported to lack recovery from faeces.¹⁰ At the higher concentration, 93% (42) of the isolates were recovered on the CAT medium whereas 69% (31) of strains were isolated on modified CCDA.

The ability of the newly formulated CAT supplement to inhibit the growth of faecal contaminants, when compared with the modified CCDA supplement, was slightly inferior to the latter. Nevertheless, the small increase in growth of other organisms—mainly *Enterobacteriaceae*—did not create difficulty in the recognition and hence the isolation of campylobacters.

This study has shown that *C upsaliensis* can be isolated from human faeces using the CAT supplement and that the latter is as efficient as the modified CCDA supplement for the isolation of other thermophilic *Campylobacter* spp. Moreover, the need to use the membrane filtration culture technique has been reduced. Further work is continuing to compare CAT medium with membrane filtration for the recovery of *C upsaliensis* from dog and cat faeces.

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