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Basis of the Superiority of Cefoperazone Amphotericin Teicoplanin for Isolating *Campylobacter upsaliensis* from Stools

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The optimum method for isolating *Campylobacter upsaliensis* from stools has not been clearly defined. In a preliminary study, cefoperazone amphotericin teicoplanin (CAT) selective medium isolated six *C. upsaliensis* strains which were not detected using modified cefoperazone charcoal deoxycholate (mCCDA). In order to identify the factors that underlie the superiority of CAT over mCCDA for isolating *C. upsaliensis*, we examined the effect of incubation time and antibiotic content of culture media on the growth of *C. upsaliensis* isolates using semiquantitative methods. The recovery of a subgroup of *C. upsaliensis* isolates from seeded stool specimens was also evaluated. Differences in growth of *C. upsaliensis* on CAT and mCCDA were modest and were not explained by the antibiotic profiles of the two media. Recovery of *C. upsaliensis* from spiked human feces on CAT was superior to that on mCCDA at lower concentrations of organisms (10^3 CFU/ml). We conclude that although CAT is more suitable than mCCDA for the isolation of *C. upsaliensis* from stools, the superiority of CAT for detecting this organism is not accounted for by the antibiotic composition of the medium.

The prevalence of *Campylobacter upsaliensis* currently may be underestimated due to the widespread use of unsuitable isolation procedures (2, 4). The poor growth of *C. upsaliensis* on modified cefoperazone charcoal deoxycholate (mCCDA), commonly used for the detection of other thermophilic campylobacters (7), has been attributed to its susceptibility to cefoperazone (2). Techniques based on membrane filtration are among the most efficient for isolating *C. upsaliensis* (1, 8, 12). However, filtration is costly and labor-intensive and may be unsuitable for samples containing small numbers of organisms. Although PCR-based (11, 13, 14, 17) and enzyme immunoassay-based (10) assays are promising, their roles for detecting *C. upsaliensis* have yet to be defined.

Using a modification of blood-free selective media containing reduced levels of cefoperazone, Aspinall et al. (2, 3) demonstrated recovery of *C. upsaliensis* on cefoperazone amphotericin teicoplanin selective medium (CAT) that was equivalent to recovery using membrane filtration and considerably superior to recovery on mCCDA. Subsequently, CAT was shown to be superior to mCCDA for the isolation of *C. upsaliensis* using a semiquantitative plating method (6). However, not all workers have found CAT to be ideal for recovering *C. upsaliensis* from clinical specimens (9), and the reasons for these contradictory results are unclear.

In a study aimed at identifying fresh clinical isolates of *C. upsaliensis* in stools of children and animals in Dublin, we noted that six *C. upsaliensis* isolates were recovered on CAT while none were recovered using incubation on mCCDA. To further investigate the reasons for the different yield of *C. upsaliensis* using these two regimens, we examined the antibiotic sensitivities of these six isolates. In addition, we characterized the effects of incubation time and antibiotic composition of media on the growth of a group of *C. upsaliensis* isolates obtained from the Laboratory Centre for Disease Control (LCDC), Winnipeg, Canada. Finally, we compared the recovery from spiked fecal samples of *C. upsaliensis* strains using these two media.

The bacteria used in this study comprised 11 *C. upsaliensis* strains obtained from the LCDC, 6 clinical isolates of *C. upsaliensis* obtained from human and animal stool specimens at Our Lady's Hospital, Crumlin, Ireland, and *C. upsaliensis* ATCC 43954. Thermophilic campylobacters were incubated at 37°C under microaerophilic conditions, generated using the CampyGen system (CN35; Oxoid Ltd., Basingstoke, Hampshire, England) to give approximately 6% oxygen and 10% carbon dioxide without evolution of hydrogen in anaerobic jars.

Media used included Columbia agar base (Oxoid CM331) containing 5% defibrinated horse blood and a *Campylobacter* blood-free selective agar base containing charcoal (Oxoid CM739). CCDA selective supplement (Oxoid), providing 32 mg of cefoperazone and 10 mg of amphotericin per liter, and CAT selective supplement (Oxoid), providing 8 mg of cefoperazone, 10 mg of amphotericin, and 4 mg of teicoplanin per liter, were added where appropriate. The in-house supplements amphotericin (Fungizone; Squibb) and teicoplanin (Targocid; Marion Merrell) were added to base agars, where indicated. MICs of cefoperazone were determined using the Etest (AB Biodisk, Solna Sweden). Brucella broth (Difco Laboratories, Detroit, Mich.) was used for suspension and dilution

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of organisms. McFarland tubes were used for standardization of inoculum (bioMerieux, Marcy l'Etoile, France). Stools used for spiking studies were first prescreened for enteric pathogens, including *C. upsaliensis*.

The original purpose of the screening study was to identify fresh clinical isolates of *C. upsaliensis* for pathogenicity studies. Over a 9-month period, a total of 2,064 stool specimens from hospitalized and nonhospitalized children with diarrhea were cultured on mCCDA and CAT agar. Stools were directly inoculated onto plates. mCCDA plates were incubated at 37°C in a microaerophilic atmosphere for 48 h in the clinical laboratory. The aim of the screening study was to achieve a maximum possible yield of *C. upsaliensis*. Therefore, CAT cultures were maintained for a longer incubation period of 96 h, as prolonged incubation time is associated with increased yield of organisms. The frequency of isolation of *C. upsaliensis* over the time period was retrospectively compared on both media. In addition, in order to increase the yield of isolates with which to work, rectal swabs from 24 asymptomatic animals (17 dogs, 4 cats, and 3 rabbits) attending a veterinary clinic were cultured on CAT agar and mCCDA for 96 h. As these samples were not processed by the clinical laboratory, the incubation period on mCCDA also was extended to 96 h. All *Campylobacter* species were fully identified using an API Campy system (API 20800; bioMerieux) or were identified at the *Campylobacter* Reference Laboratory, Public Health Laboratory Service, Central Public Health Laboratory, Colindale, United Kingdom.

Eleven strains of *C. upsaliensis* obtained from the LCDC and the type strain were cultured on Columbia blood agar without additives for 48 to 72 h at 37°C. Suspensions of each strain were made in brucella broth to a density equivalent to a number 6 McFarland standard. Ten microliters of each suspension were inoculated in duplicate on to CAT agar and mCCDA agar. This inoculum was streaked using a standard disposable 10-μl loop by the ecometric method described by Corry et al. (7). Briefly, the primary inoculum was spread using five parallel strokes, approximately 5 mm apart, over sector A. A fresh loop was used to repeat this pattern, at a 45° angle to sector A, forming sector B. This process was repeated three more times (sectors C through E). All plates were incubated in a microaeropilic atmosphere at 37°C, and growth was assessed ecometrically after 48, 72, and 96 h. The growth on each plate was expressed as the absolute growth index (AGI), depending on the number of streaks with visible growth. Growth on all five streaks (A through E) was designated an AGI of 5, growth on A through D was given an AGI of 4, growth on A through C was given an AGI of 3, and so on. A preliminary pilot study confirmed no appreciable effect on *C. upsaliensis* growth of repeated opening of gas jars compared with undisturbed incubation over 96 h (data not shown).

Etests with cefoperazone were performed on the six *C. upsaliensis* isolates recovered from stool samples during the screening study. Etests were performed in duplicate for each isolate. In order to examine the possibility that the composition of the agar base or the presence of teicoplanin could influence cefoperazone sensitivity results, each strain was subjected to Etests on blood agar base and charcoal base, both with and without 4 mg of teicoplanin per liter. Plates were dried for 30 min prior to use. Each set of plates was swab inoculated using the same suspension of organism in brucella broth at a 0.5 to 1.0 McFarland standard, according to the manufacturer's instructions for use with campylobacters. Plates were allowed to dry for 30 min at an ambient temperature prior to application of the strips. Plates were incubated in a microaerophilic environment, examined after 48, 72 and 96 h, and promptly returned to microaerophilic conditions.

The 11 LCDC *C. upsaliensis* isolates and the type strain used in ecometric plating studies were subjected to antibiotic disk sensitivity testing (30-μg cefoperazone disk). Isolates were tested in duplicate on Columbia blood agar and incubated under microaerophilic conditions.

The recovery from spiked human feces of *C. upsaliensis* on CAT and mCCDA was compared. Five strains of *C. upsaliensis* obtained from the LCDC that had been cultured on Columbia blood agar for 72 h were suspended in brucella broth to produce a turbidity equivalent to a number 6 McFarland standard. Serial 10-fold dilutions of each strain to extinction were made in brucella broth. One hundred microliters of each dilution was then added to 900 μl (1/10 dilution) of human feces and vortexed. Ten microliters of each spiked sample was plated in duplicate on CAT and mCCDA. The viable count of each dilution for each strain added to stool was determined by plating 10 μl of each dilution in duplicate on Columbia blood agar. Inocula were spread over the entire surface of the plates using sterile "hockey stick" spreaders and incubated at 37°C for 96 h in a microaerophilic environment.

Preliminary screening study of *C. upsaliensis* in humans and animals. The screening study yielded a total of six isolates of *C. upsaliensis*. These isolates comprised one human and five canine strains, identified from a total of 2,088 clinical specimens. The patient was a 10-year-old child with chronic diarrhea and abdominal pain. No other pathogens were identified in the stools of this child. The five dogs with *C. upsaliensis* in stools were asymptomatic.

All the *C. upsaliensis* strains were isolated on CAT, while none were recovered on mCCDA. The human fecal sample that yielded a *C. upsaliensis* isolate had been incubated on mCCDA for 48 h, according to our standard clinical laboratory practice, compared with 96 h of incubation on CAT. However, the five canine isolates of *C. upsaliensis* also were recovered only on CAT, despite incubation for the longer period of 96 h on both media. It was also noteworthy that of a total of 44 *Campylobacter* strains isolated during the study period, five others (four *C. jejuni* and one *C. fetus* isolate) grew only on CAT. Three percent of CAT cultures (in contrast to no mCCDA cultures) were uninterpretable due to overgrowth of fecal flora.

Comparison of *C. upsaliensis* growth using the ecometric method. In the initial screening study, human clinical specimens benefited from an incubation period on CAT that was twice that of mCCDA, raising the possibility that the differences in incubation period accounted, at least in part, for the isolation of the human *C. upsaliensis* strain. To address this question we compared the growth of *C. upsaliensis* on both media after 48, 72, and 96 h of incubation using a semiquantitative technique (the ecometric method). A preliminary pilot experiment determined that growth did not occur on either medium after 24 h of incubation, and thereafter, plates were no longer examined at this time point. *C. upsaliensis* ATCC 43954 failed to grow on both CAT and mCCDA media

TABLE 1. AGIs for *C. upsaliensis* on CAT and mCCDA using the ecometric method

Incubation time (h)	AGI	No. of isolates obtained on:	
		CAT	mCCDA
48	0	1	2
	1	4	4
	≥2	7	6
72	0	1	2
	1	2	3
	≥2	9	7
96	0	1[a]	1[a]
	1	1	2
	≥2	10	9

[a] The type strain failed to grow on the medium.

AGI = 0). Growth was supported, however, on the charcoal base without cefoperazone. For the remaining 11 isolates, CAT showed a marginal benefit over mCCDA (Table 1) with 9 and 10 isolates growing well (AGI ≥ 2) at 72 and 96 h of incubation, respectively, on CAT, compared with 7 and 9 isolates with an AGI of ≥2 on mCCDA, after the same incubation period. Colonies at all time points tended to be smaller for *C. upsaliensis* growing on mCCDA than for *C. upsaliensis* on CAT. However, all 11 isolates were successfully cultured on both media by 96 h.

These experiments also were performed using charcoal medium with amphotericin and 8 mg of cefoperazone per liter but without teicoplanin (i.e. CAT without teicoplanin) to evaluate the potential modification by teicoplanin of the growth of *C. upsaliensis*. However, there was no appreciable difference in growth in the presence or absence of teicoplanin (data not shown). Taken together, these data show that the differences in the abilities of CAT and mCCDA to support growth of *C. upsaliensis* are not great and should not on their own have accounted for the divergence in recovery of the organism from clinical specimens observed in the screening study.

Antibiotic sensitivity testing. The six clinical isolates were subjected to antibiotic sensitivity testing using Etests. Swarming of organisms coupled with hazy growth at the edge of the zone of inhibition affected precise readings of Etest results. MICs for the human isolate were variable and showed poor reproducibility. However, all the canine isolates were resistant to cefoperazone: two strains (D4 and D6) showed no zone of inhibition on any of the medium combinations (i.e., MIC > 256 mg/liter), MICs for two isolates (D1 and D5) ranged from 64 to 256 mg/liter, and the MIC for another isolate (D2) ranged from 32 to 96 mg/liter. For both the type strain and the human clinical isolate, MICs tended to be lower when the organisms were cultured on charcoal or in the presence of teicoplanin. However, the presence of teicoplanin did not increase the MIC of cefoperazone for any strain of *C. upsaliensis*. The type strain, used as a control in these experiments, was cefoperazone sensitive (MIC, 0.094 to 0.75 mg/liter), thus accounting for its failure to grow on CAT medium. It was noteworthy that all clinical isolates of *C. upsaliensis* also were successfully subcultured on mCCDA following initial isolation on CAT.

Antibiotic disk sensitivity testing of *C. upsaliensis* isolates used in the ecometric plating studies yielded similar results. The type strain was very sensitive to cefoperazone, with a radius of growth inhibition of 46 mm. However, all of the other isolates tested showed growth right up to the disk (no inhibition). Taken together, these data indicate that factors other than those relating to the concentration of cefoperazone in CAT and mCCDA account for the differences in yield of *C. upsaliensis*.

Comparison of CAT and mCCDA for the recovery of *C. upsaliensis* from spiked human feces. From the fecal specimens spiked with approximately 10^3 CFU of *C. upsaliensis* per ml all five strains tested were recovered using CAT (Table 2). However, only one of these five strains was recovered on mCCDA at this concentration. When the concentration of *C. upsaliensis* organisms in stools was increased to 10^4 CFU/ml or more, recovery of all five isolates on mCCDA was achieved. Therefore, there was approximately a 1-log difference in the sensitivity of mCCDA compared to that of CAT for the recovery of *C. upsaliensis* from seeded stool specimens. All of the strains used in the fecal spiking experiment were resistant to cefoperazone in antibiotic disk sensitivity testing. Therefore, the increased yield of *C. upsaliensis* on CAT compared with mCCDA is not explained by intermediate cefoperazone sensitivities in this group of organisms.

C. upsaliensis has been widely implicated as a potential human pathogen (4, 5, 8, 15, 16, 18). However, isolation of *C. upsaliensis* from clinical specimens has proved challenging, and this difficulty presents a substantial barrier to achieving a better understanding of its role in human disease. CAT medium was developed to contain a relatively low concentration of cefoperazone, as the presence of cefoperazone was felt likely to underlie the inefficiency of mCCDA for isolation of *C. upsaliensis* (2). However, our experiments in vitro indicate that even when *C. upsaliensis* isolates demonstrate resistance at or above the level of cefoperazone used in mCCDA and the incubation period is extended to 96 h, CAT still performs better than mCCDA. In particular, the strains that were successfully isolated from clinical samples were generally highly resistant to this antibiotic. Although the level of resistance to cefoperazone observed among *C. upsaliensis* isolates in this study was in excess of that previously reported, it is noteworthy that the MIC of cefoperazone was ≥32 mg/liter for 86% of isolates in the original study by Aspinall et al. (2). Whether all *C. upsaliensis* isolates demonstrate this level of cefoperazone resistance is unknown. However, data from this and other studies (2, 3) suggest that the sensitivity of the type strain to cefoperazone is the exception rather than the rule. Therefore, it would appear that factors other than, or in addition to, cefoperazone

TABLE 2. Comparison of recovery of *C. upsaliensis* from spiked fecal samples on CAT and mCCDA

Strain	Viable count inoculated	Recovery[a] on:	
		CAT	mCCDA
16671	4.2×10^3	1.0×10^2	0
16948	5.8×10^3	6.0×10^2	0
17228	4.7×10^3	1.0×10^3	0
17222	3.0×10^3	3.0×10^2	0
17439	8.0×10^2	3.0×10^2	50

[a] CFU/per milliliter of spiked fecal sample.

concentration underlie the superiority of CAT medium over mCCDA for isolating *C. upsaliensis*.

The other principal difference between mCCDA and CAT is the presence in CAT of teicoplanin (added in order to suppress growth of enterococci in stool samples). In this study we have examined the effects of teicoplanin on growth of *C. upsaliensis*. We found no evidence that the presence of teicoplanin in media encouraged the growth of *C. upsaliensis*, either directly or by reducing the sensitivity of the organism to cefoperazone.

The results of fecal spiking experiments in this study suggest that the improved efficiency of isolation of *C. upsaliensis* on CAT compared with that on mCCDA reflects an enhanced ability of CAT to detect small numbers of viable organisms in stool. However, it is not clear what attributes of CAT medium account for this improved yield. Our data do not point to any single component of CAT, either alone or in combination, that adequately explains the improved yield of *C. upsaliensis* observed for CAT in clinical studies. It is possible that CAT exerts a beneficial effect indirectly on isolation of this organism. We speculate that either the different antibiotic profiles of CAT and mCCDA alter some component or product of the fecal microflora, or exposure of *C. upsaliensis* to feces alters the sensitivity in vivo of *C. upsaliensis* to cefoperazone, thereby enhancing the isolation of *C. upsaliensis*. These hypotheses now warrant validation in clinical studies, as the possibility that the antibiotic composition of media may enhance target organism isolation indirectly may have relevance to culture techniques for other fastidious enteric pathogens.

Finally, data from this and previous (6) studies that CAT may be more productive than mCCDA for *Campylobacter* species in addition to *C. upsaliensis*. Because overgrowth of fecal flora may occur more commonly with CAT than mCCDA, further studies are warranted before routinely recommending CAT as an alternative selective medium for all thermophilic campylobacters. Nonetheless, in populations where *C. upsaliensis* is known to be prevalent in stools (8, 12) and in epidemiological studies of *Campylobacter*, CAT should be used in preference to other *Campylobacter* selection media.

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REFERENCES

1. **Albert, M. J., W. Tee, and A. S. Leach.** 1992. Comparison of a blood-free medium and a filtration technique for the isolation of *Campylobacter* spp. from diarrhoeal stools of hospitalised patients in Central Australia. J. Med. Microbiol. **37:**176–179.
2. **Aspinall, S. T., D. R. A Wareing, P. G. Hayward, and D. N. Hutchinson.** 1993. Selective medium for thermophilic campylobacters including *Campylobacter upsaliensis*. J. Clin. Pathol. **46:**829–831.
3. **Aspinall, S. T., D. R. A. Wareing, P. G. Hayward, and D. N. Hutchinson.** 1996. A comparison of a new *Campylobacter* selective medium (CAT) with membrane filtration for the isolation of thermophilic campylobacters including *Campylobacter upsaliensis*. J. Appl. Bacteriol. **80:**645–650.
4. **Bourke, B., V. L. Chan, and P. Sherman.** 1998. *Campylobacter upsaliensis*: waiting in the wings. Clin. Microbiol. Rev. **11:**440–449.
5. **Bourke, B., and P. Sherman.** 1999. Gastrointestinal infections in children. Curr. Opin. Gastroenterol. **15:**79–84.
6. **Corry, J. E. L., and H. I. Atabay.** 1997. Comparison of the productivity of cefoperazone amphotericin teicoplanin (CAT) agar and modified charcoal cefoperazone deoxycholate (mCCD) agar for various strains of *Campylobacter, Arcobacter* and *Helicobacter pullorum*. Int. J. Food Microbiol. **38:** 201–209.
7. **Corry, J. E. L., G. D. W. Curtis, and R. M. Baird.** 1995. Culture media for food microbiology. Prog. Ind. Microbiol. **34:**473–478.
8. **Goossens, H., L. Vlaes, M. DeBoeck, B. Pot, K. Kersters, J. Levy, P. De Mol, J. P. Butzler, and P. VanDamme.** 1990. Is "*Campylobacter upsaliensis*" an unrecognised cause of human diarrhoea? Lancet **335:**584–586.
9. **Hald, B., and M. Madsen.** 1997. Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. J. Clin. Microbiol. **35:**3351–3352.
10. **Hindiyeh, M., S. Jense, S. Hohmann, H. Benett, C. Edwards, W. Aldeen, A. Croft, J. Daly, S. Mottice, and K. C. Carroll.** 2000. Rapid detection of *Campylobacter jejuni* in stool specimens by enzyme immunoassay and surveillance for *Campylobacter upsaliensis* in the greater Salt Lake City area. J. Clin. Microbiol. **38:**3076–3079.
11. **Lawson, A. J., D. Linton, J. Stanley, and R. J. Owen.** 1997. Polymerase chain reaction detection and speciation of *Campylobacter upsaliensis* and *C. helveticus* in human faeces and comparison with culture techniques. J. Appl. Microbiol. **83:**375–380.
12. **Lindblom, G. B., E. Sjogren, J. Hansson-Westerberg, and B. Kayser.** 1995. *Campylobacter upsaliensis, C. sputorum sputorum* and *C. concisus* as common causes of diarrhoea in Swedish children. Scand. J. Infect. Dis. **27:**187–188.
13. **Linton, D., R. J. Owen, and J. Stanley.** 1996. Rapid identification by PCR of the genus *Campylobacter* and five *Campylobacter* species enteropathogenic for man and animals. Res. Microbiol. **147:**707–718.
14. **Metherell, L. A., J. M. J. Logan, and J. Stanley.** 1999. PCR-enzyme linked immunosorbent assay for detection and identification of *Campylobacter* species: application to isolates and stool samples. J. Clin. Microbiol. **37:**433–435.
15. **Mooney, A., M. Clyne, T. Curran, D. Doherty, B. Kilmartin, and B. Bourke.** 2001. *Campylobacter upsaliensis* exerts a cytolethal distending toxin effect on HeLa cells and T lymphocytes. Microbiology **147:**735–743.
16. **Patton, C. M., N. Shaffer, P. Edmonds, T. J. Barrett, M. A. Lambert, C. Baker, D. M. Perlman, and D. J. Brenner.** 1989. Human disease associated with "*Campylobacter upsaliensis*" (catalase-negative or weakly positive *Campylobacter* species) in the United States. J. Clin. Microbiol. **27:**66–73.
17. **Sails, A. D., F. J. Bolton, A. J. Fox, D. R. A. Wareing, and D. L. A. Greenway.** 1998. A reverse transcriptase polymerase chain reaction assay for the detection of thermophilic *Campylobacter* spp. Mol. Cell Probes **12:**317–322.
18. **Sandstedt, K., and J. Ursing.** 1991. Description of *Campylobacter upsaliensis* sp. nov. previously known as the CNW group. Syst. Appl. Microbiol. **14:**39–45.