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Efficient Isolation of *Campylobacter upsaliensis* from Stools

Byrne et al. (1) presented data on the superiority of cefoperazone amphotericin teicoplanin (CAT) selective medium over modified cefoperazone charcoal deoxycholate selective medium for the efficient isolation of *Campylobacter upsaliensis* from stools. There are alternatives to the use of selective media for the isolation of *C. upsaliensis*. Since 1977 we have routinely isolated campylobacters from the diarrhetic stools of pediatric patients at the Red Cross Children's Hospital, Cape Town, South Africa. In 1990, primarily for cost containment reasons, the use of antibiotic-containing selective media for *Campylobacter* isolation was discontinued in our laboratory and the Cape Town protocol was introduced. This isolation protocol was the first to combine both membrane filtration onto antibiotic-free blood agar plates and incubation in an H_2-enhanced microaerobic atmosphere (3). With the use of this protocol, the number of stool cultures positive for campylobacteria rose to 21.8% from the 7.1% previously obtained with Skirrow's and other selective media available at that time (3). Since the introduction of the Cape Town protocol we have isolated over 1,200 strains of *C. upsaliensis* from the diarrhetic and normal stools of pediatric and adult patients and from dogs, cats, and meercats (2). Our laboratory could begin to isolate *C. upsaliensis*, *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter rectus*, *Campylobacter sputorum* biovar *sputorum*, *Campylobacter hyointestinalis*, *Helicobacter fennelliae*, *Helicobacter cinaedi*, *Arcobacter butzleri*, and other campylobacteria from the stools of humans and animals only with the introduction of the Cape Town protocol. Some strains of campylobacteria are sensitive to antibiotics commonly used in selective media or have an essential requirement for an H_2-enhanced microaerobic atmosphere.

We have compared the efficacy of the filtration component of the Cape Town protocol with that of CAT selective medium for *C. upsaliensis* isolation from 300 consecutive diarrhetic stool samples from gastroenteritis patients at the Red Cross Children's Hospital (Table 1). The antibiotic-free filtration and CAT isolation plates were incubated under identical conditions, in an H_2-enhanced microaerobic atmosphere at 37°C. *Campylobacter*, *Helicobacter*, and *Arcobacter* isolates were identified by recognized phenotypic and biochemical criteria. The data in Table 1 indicate that with filtration onto antiobiotic-free plates, 20.3% of the stools were positive for campylobacteria, while with the use of CAT selective plates only 4.7% of the same stools were positive for campylobacteria. Both methods were equally efficient for the isolation of *Campylobacter coli* and *A. butzleri*; however, filtration was superior to CAT selective medium for all other campylobacteria isolated. *Campylobacter jejuni* subsp. *doylei*, *H. fennelliae*, *C. hyointestinalis*, and *C. concisus* strains were isolated with filtration but were not isolated with CAT media. Sixteen strains of *C. jejuni* subsp. *jejuni* were isolated with filtration, whereas nine strains were isolated with CAT medium. Eleven *C. upsaliensis* strains were obtained with filtration, but only a single *C. upsaliensis* strain was obtained with CAT medium. Generally, colonies of *C. upsaliensis* and other campylobacteria on the antibiotic-free blood agar plates used in the Cape Town protocol were larger, more prominent, and faster growing (visible growth after 2 to 4 days) than those on the CAT plates.

TABLE 1. Efficiency of filtration versus that of CAT selective medium for isolation of *C. upsaliensis* and related organisms from 300 consecutive diarrhetic stools of patients at the Red Cross Children's Hospital

Organism(s)	No. of isolates obtained by use of:	
	Filtration	CAT selective medium
C. concisus	21	
C. jejuni subsp. *jejuni*	16	9
C. upsaliensis	11	1
C. coli	3	3
C. jejuni subsp. *doylei*	2	
C. hyointestinalis	1	
A. butzleri	1	1
H. fennelliae	4	
Helicobacter spp.[a]	2	
Total isolates[b]	61	14

[a] *Helicobacter* spp. that could not be fully identified to species level.
[b] The percentages of stool cultures that were positive for *C. upsaliensis* and related organisms were as follows: with the use of filtration, 20.3%, and with the use of CAT selective medium, 4.7%.

Byrne et al. (1) state that membrane filtration is costly and labor intensive. We do not agree, as the Cape Town protocol, which has been in continuous use over the last 11 years, has proved to be a simple, efficient, and cost-effective alternative to the use of antibiotic-containing selective media for the isolation of *C. upsaliensis* and other campylobacteria from stool. The underdetection of *C. upsaliensis* and other campylobacteria in the stools of gastroenteritis patients is an important diagnostic problem, and application of the Cape Town protocol may help alleviate this concern.

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Authors' Reply

We are most grateful to Drs. Lastovica and Le Roux for their interest in our recent paper on *Campylobacter upsaliensis* isolation. During the course of experiments aimed at identifying the basis of the differences in productivity between two widely available *Campylobacter* selective media for isolating *C. upsaliensis* (2), we also examined the effect of the Cape Town protocol conditions on the growth of 15 of our isolates (unpublished data). When the growth of the 15 isolates was compared to that observed with conventional incubation using the

CampyGen system, we found that 9 *C. upsaliensis* isolates showed reduced growth (using the ecometric plating system) and 3 isolates failed to grow at all under the Cape Town protocol conditions.

Nevertheless, the Cape Town protocol undoubtedly has contributed greatly to the rate of isolation of campylobacters and related organisms at the Red Cross Children's Hospital, Cape Town, South Africa. The findings presented by Lastovica and Le Roux concerning the superiority of this protocol compared with the use of cefoperazone amphotericin teicoplanin selective medium for isolation of *C. upsaliensis* in their hands indicates the potential for application of their isolation methodology in investigating the epidemiology of enteric *Campylobacter* infection. Studies by other investigators comparing the productivities of the Cape Town protocol and of selective media among populations with lower prevalences of campylobacters clearly are warranted.

However, for the present, we feel that our own findings, together with concerns regarding the sensitivity of filtration methods for low numbers of organisms (1, 3), the possible biohazard of high hydrogen levels (4), and the perceived awkwardness of filtration methodology, pose a substantial barrier to the attractiveness of filtration-based techniques in the clinical laboratory setting.

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