JOURNAL OF CLINICAL MICROBIOLOGY, July 2000, p. 2798–2799 Vol. 38, No. 7
0095-1137/00/$04.00+0

Efficient Isolation of Campylobacteria from Stools

Engberg et al. (2) compared selective media and filtration for the efficient isolation of campylobacteria from stools. Since 1977, we have routinely isolated campylobacters from the diarrhetic stools of pediatric patients at the Red Cross Children's Hospital in Cape Town, South Africa. In 1990, the use of antibiotic-containing plates was discontinued, and the Cape Town protocol, the first to combine both membrane filtration onto antibiotic-free blood agar plates and incubation in an H_2-enhanced microaerobic atmosphere (3), was introduced.

With the use of this protocol, stool cultures positive for campylobacteria rose to 21.8% from the 7.1% previously obtained with Skirrow's medium and other selective media used in conjunction with conventional microaerobic incubation (3). Our laboratory could only begin to isolate *Campylobacter upsaliensis*, *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter rectus*, *Campylobacter sputorum* biovar *sputorum*, *Helicobacter fennelliae*, *Helicobacter cinaedi*, "*Helicobacter rappini*", and *Arcobacter butzleri* from stool samples with the use of the Cape Town protocol. Strains of these species are sensitive to antibiotics commonly used in selective media or have an essential requirement for an H_2-enhanced microaerobic atmosphere.

With a combination of filtration and selective media, Engberg et al. (2) documented an isolation rate of 4.8% for eight species of campylobacteria from diarrhetic stools. The Cape Town protocol, utilizing only membrane filtration, yielded an isolation rate of 21.1% for 16 species or subspecies of *Campylobacter* and related organisms (Table 1). Differences in colonial morphology on primary isolation and subsequent biochemical, serological, or molecular confirmation indicated that 16.2% of South African children suffering gastroenteritis had coinfections with up to five different species of campylobacteria (3).

TABLE 1. Distribution of *Campylobacter* and related species isolated from 19,535 diarrhetic stools of pediatric patients at the Red Cross Children's Hospital, Cape Town, South Africa, from 1 October 1990 to 29 February 2000

Species and subspecies	Specimens	
	No. positive	% Positive
C. jejuni subsp. *jejuni*, biotype 1[a]	1,166	28.29
C. concisus	971	23.55
C. upsaliensis	948	23.00
C. jejuni subsp. *doylei*	378	9.17
H. fennelliae	260	6.31
C. coli	119	2.87
C. jejuni subsp. *jejuni*, biotype 2[a]	115	2.79
C. hyointestinalis	53	1.29
H. cinaedi	42	1.01
CLO/HLO[b]	35	0.85
Arcobacter butzleri	16	0.39
C. fetus subsp. *fetus*	7	0.17
"*H. rappini*"	4	0.10
C. lari	2	0.05
C. curvus	2	0.05
C. rectus	2	0.05
C. sputorum biovar *sputorum*	2	0.05
Total	4,122	100.00

[a] Biotype of Skirrow and Benjamin (4).
[b] CLO/HLO, *Campylobacter* or *Helicobacter* organisms that could not be fully characterized.

Discrepancy in the isolation rate and numbers of species detected between the Danish and South Africa studies may reflect geographical differences in the prevalence of various campylobacteria, the nature of infection in these countries, or other factors. A final hydrogen concentration of 3% was advocated by Engberg et al. (2) as being optimal. Use of Oxoid BR 38 GasPaks with no catalyst in the Cape Town protocol generates a hydrogen concentration of greater than 40%. This increased level of hydrogen, combined with strain variation in hydrogen requirements, may contribute to the higher isolation rate of the Cape Town protocol.

Campylobacter jejuni and *Campylobacter coli* comprise about one-third of the campylobacteria routinely isolated in Cape Town (Table 1). We concur with Engberg et al. (2) that campylobacteria other than *C. jejuni* and *C. coli* are responsible for gastroenteritis and are undetected by inappropriate techniques. We also agree that *C. concisus* is an important opportunistic pathogen of very young children (1). The pathogenic potential, reservoirs, and modes of transmission of these non-*C. jejuni* or non-*C. coli* species have yet to be fully determined (1).

Engberg et al. (2) recommend a combination of both membrane filtration and selective media for optimal isolation of campylobacteria from stool. They also state that membrane filtration is costly, labor-intensive, and less sensitive than selective media. We do not agree, as the Cape Town protocol in our laboratory over the last decade has proved to be a simple, efficient, and cost-effective alternative to the use of selective media.

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Author's Reply

Lastovica and le Roux raise a number of important issues regarding the isolation and prevalence of "campylobacteria" that were mentioned in our study (2). We agree that discrepancies in the isolation rate and number of species detected between the Danish and South African studies may reflect geographical differences in the prevalence of various campylobacteria, differing sources, and routes of transmission of

campylobacterial species in these countries. However, other factors should also be considered. These include the number of fecal samples tested and differences in study populations. We processed 1,376 stools primarily from outpatients and from all age groups (2), whereas the South African material consisted of 19,535 stools exclusively from pediatric patients admitted to hospital. The importance of comparable age groups is reflected by the differential distribution of *Campylobacter concisus* among patients of different ages (2) and, moreover, by the high proportion (66%) of *C. concisus* strains in children that were isolated from patients less than 2 years of age. Thus, differences in isolation rate may be biased by sample size, severity of infections, and age groups tested.

The influence of identification procedures on the numbers of species detected between our laboratories must also be considered. In our initial studies, we found that strains of *Sutterella wadsworthensis* frequently resembled *C. concisus* in phenotypic tests. We used a polyphasic approach to identification in our study, and this has been recommended for confirmation of, especially, non-*C. jejuni*, non-*C. coli* species (3). Lastovica and le Roux do not mention in detail which identification methods are used in their laboratory, but it is possible that some of their *C. concisus* strains could be misclassified *S. wadsworthensis* isolates. The fact that these authors have been unable to identify 35 strains to the species level emphasizes the complex issue of campylobacterial identification. The identity of these as-yet-unclassified organisms could emphasize further differences or, conversely, similarities between the species distribution between our laboratories.

An atmosphere enriched with hydrogen (6 to 7%) is generally advocated for the isolation of hydrogen-requiring *Campylobacter* spp. (3, 4). The efficacy of the Cape Town protocol for isolating these and other fastidious species is well documented, but most laboratories, including our own, are wary of using growth atmospheres containing such a high proportion of flammable hydrogen. In our study, with a final hydrogen content of 3%, we were able to isolate a high number of hydrogen-requiring *C. concisus* strains; this allowed us to suggest that this hydrogen content, if combined with an appropriate nonselective medium, may suffice. We did not advocate this hydrogen level as being optimal, as mentioned by Lastovica and le Roux. However, our suggestion is supported by 23% of our *C. concisus* strains being recovered after only 2 days of incubation. This is in contrast to the literature (3) in which *C. concisus* is reported to need 4 days of incubation for their isolation. Moreover, when subcultures of *C. concisus* were performed, with the same medium and atmosphere, sufficient growth could be achieved within just 24 h of incubation. Finally, it is notable that eight and one *C. concisus* strains were isolated by using Skirrow's and modified charcoal cefoperazone deoxycholate agar (mCCDA) selective media, respectively, suggesting that at least some strains of the species may be less fastidious than previously considered. However, what is more important is the clarification of the pathogenic potential of this species.

Lastovica and le Roux state that the use of the Cape Town protocol for isolation of campylobacteria (i.e., membrane filtration method in conjunction with high atmospheric hydrogen levels) was a contributing factor in their high isolation rate (21.8%) of campylobacteria. It is interesting to postulate whether or not this admittedly impressive isolation rate could be improved further by the use of appropriate selective media. Skirrow's medium is the only selective agar named by the authors, and many studies have shown that this medium is less sensitive than more recently developed media such as mCCDA for isolating *C. jejuni*, *C. coli*, and *C. lari* (reviewed by Corry et al. [1]). Given these data, can the authors authoritatively claim that they are detecting all possible cases of infection due to campylobacteria? Certainly it is generally recognized that the membrane filter method is relatively insensitive for isolating campylobacteria (3, 4), and while the use of high hydrogen levels may in part compensate for this deficiency, the underdiagnosis of some thermophilic campylobacter infections by reliance on the filter method remains a possibility.

It should also be noted that the membrane filter method relies on the ability of motile bacteria small enough to pass through the filter pore size onto the unselective growth medium. Nonmotile species such as *C. gracilis* or species with cell bodies too large to pass through the filter (*Anaerobiospirillum* spp.) or with growth requirements not met by those provided by the Cape Town protocol (e.g., strict anaerobes such as *C. showae*) would not therefore be isolated with this method. We emphasize here that these species, and many others, would also be undetected using mCCDA, cefoperazone-amphotericin-teicoplanin (CAT) agar, and Skirrow's medium, and we concur with Lastovica and le Roux that underdetection of campylobacteria in gastroenteritis is an important diagnostic issue.

Our results emphasize that at present, no single method will successfully isolate all campylobacteria. This is in line with generally accepted recommendations for a comprehensive isolation strategy, where filtration should be used to complement culturing on selective plating media and not as a replacement (4). The development of a single, simple, and cost-effective method of isolating all campylobacteria remains an important goal for clinical microbiologists.

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