Comparison of two simple microaerobic atmospheres for cultivation of thermophilic campylobacters

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Abstract

A comparison of a modification of the spirit burn method and one based on the Fortner principle was carried out on 3474 faecal samples from patients with diarrhoea. Of the 96 isolates of *Campylobacter*, 47 showed equal abundance of growth, regardless of the method used. By using the spirit burn method, however, growth of 33 isolates was significantly increased, and 18 out of those 33 isolates grew only in the spirit burn method atmosphere. The spirit burn method is more effective than the one based on the Fortner principle, it is easy to perform, quick, and cheap.

Optimal atmosphere for the growth of thermophilic campylobacters is between 5% and 10% oxygen and between 1% and 10% carbon dioxide. This may be produced in several ways. The evacuation-replacement system and gas generating envelopes are the method of choice.¹ In many developing countries, however, resources do not allow these methods to be used and either a candle jar or a method based on the Fortner principle is used instead.² Ribeiro *et al* first reported an improved version of the candle extinction jar in which methylated spirit is burnt instead of a candle.³ We report here a comparison of this method with one based on the Fortner principle.

Methods

We cultured a total of 3474 faecal samples from patients with diarrhoea. Each faecal specimen was plated in duplicate on Butzler's Virion medium.⁴ One plate was incubated in an atmosphere with the oxygen lowered by a method based on a modified Fortner's principle (MFP)

in which culture plates are sealed in a plastic bag with another plate seeded with *Escherichia coli*.² The other plate was placed in a jar where the oxygen was lowered by a modification of the spirit burn method.⁵ Briefly, 0.5 ml of spirit is burnt in a 9 cm glass Petri dish, placed on the topmost culture plate a few seconds before replacing the lid of the 2.5 l stainless steel jar. On cooling, a partial vacuum is formed that keeps the lid firmly in place, provided there is a proper seal.

After 48 hours of incubation at 42°C campylobacters were identified by morphology, Gram stain, positive catalase and oxidase reactions, susceptibility to nalidixic acid (30 µg discs) and resistance to cephalothin (30 µg discs). The distinction between *C jejuni* and *C coli* was determined by hippurate hydrolysis.⁶ Reference strains *C coli* NCTC 11353 and *C jejuni* NCTC 11168 were used as controls in the identification tests.

Results

Campylobacters were isolated from 96 (2.8%) of the 3474 specimens. Of these isolates, 50 were *C jejuni* (52%) and 46 were *C coli* (48%) (table). Of the 96 campylobacter isolates, 47 showed equal abundance of growth regardless of the method used (47 strains). By using the spirit burn method, however, the growth of 33 *Campylobacter* strains was significantly increased, and 18 out of those 33 isolates grew only in the spirit burn method atmosphere; only one strain failed to grow.

Conclusion

The atmosphere suitable for the growth of campylobacters can be obtained in several ways. The method of choice greatly depends on its relative cost. More sophisticated procedures, such as disposable gas generating envelopes and evacuation replacement techniques, are often not available in developing countries. Therefore, several simple, economical methods have been devised. Ribeiro *et al*³ have shown that incubation of campylobacters in a candle jar is inferior to incubation in an atmosphere produced by the spirit burn method. Furthermore, they observed that evacuation of a jar coupled with the provision of carbon dioxide was as efficient as the spirit burn method. In our study the spirit burn method was more reliable than that based on

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Semiquantitative analysis of campylobacter growth using the spirit burn method and the modified Fortner's principle

	Spirit burn method				Total
	Heavy growth	> 5 colonies	1-5 colonies	0	
Modified Fortner's principle					
+++	36	9	—	—	45
++	12	3	6	—	21
+	—	3	8	1	12
0	—	9	9	—	18
Total	48	24	23	1	96

the Fortner principle. The method is very easy to perform and it is quick and cheap. It can be recommended for use in those laboratories where more costly supplies are limited.

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