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Recovery of campylobacter from human faeces stored at 4 °C

C. LADRÓN DE GUEVARA, M. T. PÉREZ-POMATA, A. AGULLA, F. J. MERINO, P. A. VILLASANTE AND A. C. VELASCO*

Servicio de Microbiología, Hospital 'La Paz', Castellana, 261, Madrid 28046, Spain

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SUMMARY

Six hundred and thirteen fresh diarrhoeal faeces were inoculated on Skirrow blood agar (SK), on Preston blood free agar (PBF), and in Campy-thioglycolate broth (CT). After 24 h of storage at 4 °C, specimens were again inoculated on SK and PBF, and in Campylobacter enrichment broth (CEB). CT tubes were placed overnight at 4 °C. Plates and CEB tubes were incubated at 43 °C in microaerophilic conditions. A total of 68 specimens was positive for campylobacter on direct plating. Sixty-four of them were also recovered after subculturing from CT, and only 51 from CEB. Delayed inoculation of plates after storage of samples at 4 °C yielded 57 isolates. The storage of faeces at 4 °C for 24 h significantly reduces the number of campylobacter isolates. When samples are not plated immediately we recommend inoculating a CT tube maintained at 4 °C overnight as a holding medium.

INTRODUCTION

Campylobacter is a frequent aetiological agent of bacterial diarrhoea all over the world (Blaser *et al.* 1979; Blaser, Feldman & Wells, 1982; Velasco *et al.* 1984; Walder, 1982). For the isolation of this microorganism, it is generally recommended that stool samples are inoculated onto appropriate media as soon as possible or, if they are not processed immediately, to store them at 4 °C for a maximum of 24–48 h (Goosens *et al.* 1984; Morris & Patton, 1985). Several authors have shown that storage at this temperature allows the recovery of campylobacter from faeces and other biological milieus even after 3 weeks (Blankenship & Craven, 1982; Blaser *et al.* 1980; Grant, Richardson & Bokkenheuser, 1980; Tanner & Bullin, 1977; Svedhem, Kaijser & Sjögren, 1981) although other authors have drawn attention to the possibility of cold injury on campylobacter cells, especially when cultured on antibiotic-containing media (Humphrey & Cruickshank, 1985). The aim of the present study was to assess the recovery of campylobacter in human faeces after storage at 4 °C overnight.

MATERIALS AND METHODS

From August 1986 to June 1987, 613 unselected diarrhoeal faeces from different patients were processed on arrival at the laboratory and then refrigerated for

* Correspondence to Dr A. C. Velasco.

Table 1. *The isolates of campylobacter obtained by the different procedures used*

Procedures†	No. of isolates* SK only	PBF only	On both	P‡	Total (on SK or PBF)
IP	1	3	64	NS	68
DP	3	7	47	NS	57
CEB	1	2	48	NS	51
CT	0	6	58	0·01	64
DP/CEB	3	4	55	NS	62
DP/CT	0	5	61	0·02	66

* SK, Skirrow blood agar; PBF, Preston blood free agar.

† IP, immediate plating; DP, delayed plating; CEB, campylobacter enrichment broth; CT, Campy-thioglycolate broth; DP/CEB, delayed plating and/or after campylobacter enrichment broth; DP/CT, delayed plating and/or after Campy-thioglycolate broth.

‡ McNemar paired sample test; NS, Not significant.

24 h. A suspension of each fresh specimen diluted in saline was immediately plated on Skirrow blood agar (SK) (Oxoid Ltd, Basingstoke, England) (Skirrow, 1977) and on Preston blood free agar (PBF) (Oxoid) (Hutchinson & Bolton, 1984), and inoculated in Campy-thioglycolate broth (CT) (Blaser *et al.* 1979). Plates were incubated at 43 °C in microaerophilic conditions (5% O_2 and 10% CO_2) provided by a Gas Generating kit and Anaerobic Catalyst (Oxoid), for a 24 h period; CT tubes were kept overnight at 4 °C.

A further saline suspension was made from the faeces stored for 24 h at 4 °C and both plates and campylobacter enrichment broth (CEB) (Martin *et al.* 1983) were then inoculated. Plates and CEB tubes were incubated at 43 °C in microaerophilic conditions as above for a 24 h period. More plates of SK and PBF were inoculated from each liquid medium after incubation at 43 °C (CEB tubes) or cold storage (CT tubes).

Plates showing no growth were incubated up to 48 h. Identification of suspect colonies was made following standard criteria (Morris & Patton, 1985). A *Campylobacter jejuni* strain was inoculated daily in SK and PBF plates, for quality control.

RESULTS

The results are summarized in Table 1 and in Fig. 1. A total of 68 specimens was positive for campylobacter on immediate plating either on PBF, on SK or both, but delayed plating after storage of samples at 4 °C yielded only 57 isolates (P = 0·0009 by the McNemar paired sample test). No significant difference was found between the number of isolates obtained on PBF and on SK on direct plating. A total of 64 specimens was positive for campylobacter after subculturing from CT, and only 51 from CEB. After CT storage the isolation rates on the two plating media were significantly different (P = 0·01). Statistically significant differences were found between the numbers of isolates obtained on immediate plating and those recovered after CT (P = 0·045). The number of campylobacter isolates recovered from CT and/or delayed plating (66) was significantly different from that with delayed plating only (57) (P = 0·003). A total of 62 specimens was

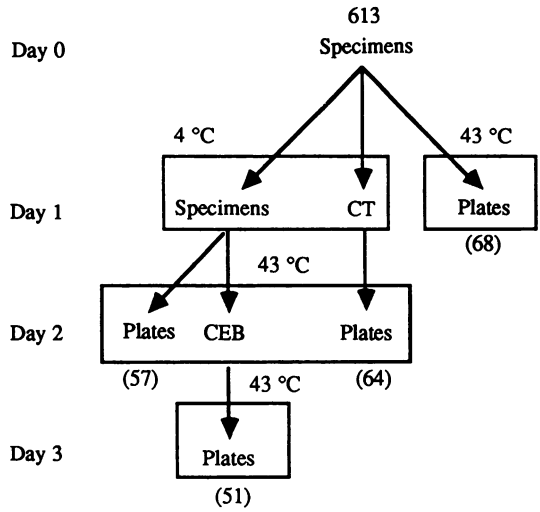

Fig. 1. Summary of procedures and results. Figures in parentheses represent isolates of campylobacter.

positive for campylobacter on delayed plating and/or in CEB, showing a slight improvement when compared with delayed plating only ($P = 0{\cdot}02$).

DISCUSSION

Previous reports have pointed out the usefulness of transport and enrichment media incubated at different temperatures (Martin *et al.* 1983; Wang *et al.* 1983) for recovering campylobacter. Some authors have contended that enrichment media are useful only when specimens contain few viable organisms or when the seeding of samples is delayed, but not so useful when faeces are processed immediately (Agulla *et al.* 1987; Hutchinson & Bolton, 1983). Many studies have been made using animal and food samples (Doyle & Roman, 1982; Luechtefeld *et al.* 1981; Rothemberg, Stern & Westhoff, 1984; Sjögren, Lindblom & Kaijser, 1987) but, as far as we know, rarely with clinical specimens from patients with acute diarrhoea (Sjögren, Lindblom & Kaijser, 1987).

CT has been previously used as a holding or enrichment medium (Chan & Mackenzie, 1986; Rubin & Woodard, 1983; Wang, Blaser & Cravens, 1978) either at 4 °C or incubated at 43 °C, the latter with poor results (Agulla *et al.* 1987; Luechtefeld *et al.* 1981). In the present study we have found little difference between the number of isolates yielded by direct plate inoculation of fresh specimens and those recovered after cold storage in CT. This difference was even smaller when the 68 isolates obtained by immediate inoculation were compared with the 66 isolates recovered either from CT or from plates inoculated after storing the samples at 4 °C. On the other hand, a significant difference was found between isolates obtained by delayed plating on solid media only, and isolates using this method plus those recovered by subculturing from CT (57 vs. 66), thus indicating that CT is useful for preserving the viability of campylobacter when immediate inoculation of fresh samples is not possible.

In contrast with previous reports (Martin *et al.* 1983), we have found few advantages in using CEB as an enrichment medium for the recovery of campylobacter from human faeces stored overnight at 4 °C, as 1 out of every 10 isolates were not recovered when this method was added to delayed plating of samples.

More isolates were obtained on PBF than on SK but statistically significant differences were not found except for isolates recovered after storage on CT. As previously reported, this might be due to contaminating faecal flora masking campylobacter colonies on SK (Merino *et al.* 1986) or to sublethal cold injury of campylobacter cells which could have increased sensitivity to certain antibiotics contained in CT and SK (Humphrey, 1986; Ng, Stiles & Taylor, 1985; Ray & Johnson, 1984).

Although cold storage has not been considered to affect the recovery of campylobacter from clinical specimens (Blaser *et al.* 1980; Tanner & Bullin, 1977), our results show that the storage of human faeces at 4 °C for 24 h, significantly reduces the number of isolates as only 57 campylobacter were obtained after cold storage compared with 68 isolates from fresh specimens.

Enrichment by CEB slightly enhances the number of isolates when compared with the culture of refrigerated samples, but delays the clinical report for 24 h.

It is essential to emphasize that one out of every six specimens positive for campylobacter did not yield this organism after cold storage overnight. For this reason, when samples are not plated immediately we recommend inoculating a CT tube maintained at 4 °C overnight as a holding medium that should be subcultured on PBF.

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