

Comparative Efficacy of Seven Selective Media for Isolating *Campylobacter jejuni*

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Diarrheal stools from 263 patients were inoculated on seven selective media: Butzler selective medium, Blaser medium, Skirrow blood agar, Preston campylobacter selective medium, Preston campylobacter blood-free medium, Butzler Virion medium, and modified Preston medium (with amphotericin B [2 mg/liter]). A similar number of *Campylobacter jejuni* strains were isolated from all the media studied; nevertheless, the presence of competing fecal flora (FF) made the detection of suspect colonies difficult. Preston campylobacter blood-free medium with cefoperazone yielded the greatest number of *C. jejuni* isolations, and contaminating FF grew in only 9% of the plates showing *C. jejuni* growth; all the other media allowed the abundant growth of other FF, regardless of whether *C. jejuni* was isolated from them or not.

Campylobacter jejuni is a common cause of diarrhea all over the world (1). In Spain, it is the most frequent etiologic agent in bacterial diarrhea in the winter and spring (9).

The standard method for isolating *C. jejuni* from human stools is the inoculation of samples on selective plates incubated at 42 to 43°C in a microaerophilic atmosphere. We evaluated seven of the many available media for the isolation of *C. jejuni*.

During a period of 7 months, 263 fecal samples were selected from different patients with acute diarrhea and no underlying disease. Emulsified stools in saline solution were inoculated on the following media, which are described elsewhere: Butzler selective medium (BU; 4), which contains bacitracin, novobiocin, cycloheximide, colistin, and cefazolin (Oxoid Ltd., Basingstoke, England); Blaser medium (BL), also known as Campy BAP (2), prepared with vancomycin, trimethoprim, polymyxin B, cephalothin, and amphotericin B (Oxoid); Skirrow blood agar (SK; 8), which contains vancomycin, trimethoprim, and polymyxin B (Oxoid); Preston campylobacter selective medium (PC) with trimethoprim, polymyxin B, rifampin, and cycloheximide (Oxoid) (3); Preston campylobacter blood-free medium (PBF) with cefoperazone (Oxoid) (6); Butzler Virion medium (BV), which contains cefoperazone, rifampin, colistin, and amphotericin B (Institute Virion, Zurich, Switzerland) (5); and modified Preston medium (MP) made up with nutrient broth no. 2 (Oxoid), 7% defibrinated horse blood, cefoperazone (32 mg/liter), amphotericin B (2 mg/liter), and campylobacter growth supplement FBP (Oxoid).

The media were incubated at 43°C for 24 h in a microaerophilic atmosphere (5% O₂, 10% CO₂) provided by a gas-generating kit and anaerobic catalyst (Oxoid). Plates showing no growth were incubated for a further 24 h. The growth of contaminating fecal flora (FF), which might interfere with the detection of *Campylobacter* colonies, was recorded. A control strain was inoculated daily on each medium.

At 24 h of incubation, *C. jejuni* showed a varied morphology from one medium to another and even within the same medium; morphology ranged from the characteristic mucoid, transparent, nonhemolytic, flat, and confluent colonies to

very small, convex, opaque, and grayish colonies. *C. jejuni* was isolated on at least one of the seven media from 46 of the 263 samples tested. The number of isolates on each medium was: 44 on BU, 43 on BL and SK, 42 on PC, 46 on PBF, and 45 on BV and MP. Visible growth was not detected until 48 h, twice on PBF and once each on PC, SK, and BV. These results, as well as the growth of competing FF on the seven different media tested, are shown in Table 1.

A similar number of *C. jejuni* strains were isolated from all the media studied; nevertheless, the presence of other FF made the detection of suspect colonies difficult and increased the time spent in reading every plate.

PBF yielded the greatest number of *C. jejuni* isolations, and competing FF grew in only 9% of the plates showing *C. jejuni* growth. On this medium, most of the contaminants observed were yeasts, which grew mainly after 48 h of incubation; they usually did not mask the characteristic morphology of *C. jejuni*, and the addition of amphotericin B therefore did not seem to be necessary. It is worth noting that most *C. jejuni* isolates (93%) were recovered after 24 h of incubation in PBF; this was probably due to the careful examination of plates after 24 h and Gram staining of all colonies, regardless of how small or atypical they were.

TABLE 1. Growth of *C. jejuni* and other FF on seven selective media

Medium ^a	Growth of <i>C. jejuni</i> (no. of isolates)		No growth of <i>C. jejuni</i> (no. of isolates)	
	Pure culture	Other FF growth	Sterile	Other FF growth
PBF	42 ^b	4	148	69
PC	5 ^c	37	34	187
BL	3	40	5	215
BU	16	28	82	137
SK	11 ^c	32	22	198
BV	29 ^c	16	126	92
MP	30	15	91	127

^a PBF, Preston campylobacter blood-free medium; PC, Preston campylobacter selective medium; BL, Blaser medium; BU, Butzler selective medium; SK, Skirrow blood agar; BV, Butzler Virion medium; MP, modified Preston medium.

^b Growth from two samples was at 48 h.

^c Growth from one sample was at 48 h.

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BV and MP also yielded good isolation rates, although they were less efficient in suppressing the growth of other FF. On the other hand, most of the plates of PC, BL, BU, and SK allowed the abundant growth of contaminating FF, regardless of whether *C. jejuni* was isolated from them or not; this made screening difficult.

Incubation for a further 24 h of the plates which showed no growth after overnight incubation did not lead to a worthwhile improvement in *C. jejuni* isolation rates but increased the growth of competing FF and, consequently, the difficulty of screening.

Cefoperazone seems to be the antimicrobial supplement of choice for *C. jejuni* selective media (7). PBF, BV, and MP contain this antimicrobial agent, and all of them yielded better isolation rates and were also more selective. The presence of blood, as in BV and MP, favored the development of competing FF, compared with blood-free media such as PBF. We agree with Hutchinson and Bolton (6) in recommending the use of PBF for the isolation of *C. jejuni* from human feces because of the greater ease of detection of suspect colonies, as well as that of medium preparation, and the cost saved by not using blood.

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LITERATURE CITED

1. Blaser, M. J., and L. Barth Reller. 1981. *Campylobacter* enteritis. N. Engl. J. Med. 305:1444-1452.
2. Blaser, M. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, L. Barth Reller, and W. L. L. Wang. 1979. *Campylobacter* enteritis: clinical and epidemiological features. Ann. Intern. Med. 91:179-185.
3. Bolton, F. J., and L. Robertson. 1982. A selective medium for isolating *Campylobacter jejuni/coli*. J. Clin. Pathol. 35:462-467.
4. Butzler, J. P., and M. B. Skirrow. 1979. *Campylobacter* enteritis. Clin. Gastroenterol. 8:737-765.
5. Goosens, H., M. De Boeck, and J. P. Butzler. 1983. A new selective medium for the isolation of *Campylobacter jejuni* from human faeces. Eur. J. Clin. Microbiol. 2:389-394.
6. Hutchinson, D. N., and F. J. Bolton. 1984. Improved blood free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J. Clin. Pathol. 37:956-957.
7. Karmali, M. A., A. E. Simor, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane. 1986. Evaluation of a blood-free, charcoal-based, selective medium for the isolation of *Campylobacter* organisms from feces. J. Clin. Microbiol. 23:456-459.
8. Skirrow, M. B. 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. 2:9-11.
9. Velasco, A. C., M. L. Mateos, G. Más, A. Pedraza, M. Díez, and A. Gutiérrez. 1984. Three-year prospective study of intestinal pathogens in Madrid, Spain. J. Clin. Microbiol. 20:290-292.