JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1986, p. 840–843
0095-1137/86/110840-04$02.00/0

Vol. 24, No. 5

Modified Selective Medium for Isolation of *Campylobacter* spp. from Feces: Comparison with Preston Medium, a Blood-Free Medium, and a Filtration System

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Received 17 April 1986/Accepted 5 August 1986

Our previously described (H. Goossens, M. De Boeck, and J. P. Butzler, Eur. J. Clin. Microbiol. 2:389–393, 1983) selective medium, consisting of cefoperazone (15 mg/liter), rifampin (10 mg/liter), colistin (10,000 IU/liter), and amphotericin B (2 mg/liter) (medium M1), for the isolation of *Campylobacter jejuni* and *Campylobacter coli* from stool specimens was modified as follows: cefoperazone (30 mg/liter), rifampin (10 mg/liter), and amphotericin B (2 mg/liter) (medium M2). A comparative study of the isolation of *Campylobacter* spp. from stool specimens was carried out with medium M1; medium M2; a selective blood-free medium consisting (per liter) of charcoal (4 g), ferrous sulfate (0.25 g), sodium pyruvate (0.25 g), casein hydrolysate (3 g), sodium deoxycholate (1 g), nutrient broth no. 2 (25 g), agar (12 g), and cefoperazone (32 mg) (medium M3); and Preston medium containing (per liter) trimethoprim (10 mg), rifampin (10 mg), polymyxin B (5,000 IU), and cycloheximide (100 mg) (medium M4). We also included a filtration system in which membrane filters were applied directly to the surface of the nonselective blood-free medium distributed in small petri dishes. A total of 5,276 stool specimens were tested: 2,788 stool specimens were tested on M1 and M3 in study 1; 2,488 stool specimens were inoculated on the four selective media in study 2, and the last 986 specimens of the 2,488 were tested in parallel with the filtration system. In study 2, 128 *Campylobacter* strains were isolated from 126 different patients; 85.0, 88.3, 82.5, and 66.6% of these strains were isolated on M1, M2, M3, and M4, respectively. No contaminating fecal flora was found on 65.4, 70.7, 62.4, and 40.3% of the M1, M2, M3, and M4 plates, respectively. Furthermore, *C. coli* was found to be more susceptible to antibiotics present in the selective media, particularly colistin and polymyxin B, than was *C. jejuni*. We therefore recommend M2 for the isolation of *Campylobacter* spp. Finally, the filtration method was found to be easy and cheap; although the sensitivity was low, this method allowed the isolation of new *Campylobacter* spp. which seem to be associated with diarrhea.

Campylobacter jejuni and *Campylobacter coli* are now recognized as major enteric pathogens as a result of the development of selective isolation methods by Butzler et al. (6, 7), Skirrow (17), Blaser et al. (3), Bolton and Robertson (5), and Goossens et al (8). These selective antibiotic combinations are added to blood-containing basal media. More recently (4), a charcoal-based, blood-free medium for the isolation of *Campylobacter* organisms from feces was described. In this study, we modified our new selective medium (8) and compared both of our media with Preston medium (5), *Campylobacter* blood-free selective medium (Oxoid Ltd.), and a modified filtration system in which membrane filters are applied directly to the surfaces of agar plates (19).

MATERIALS AND METHODS

Media. Medium M1, known as Butzler's medium Virion, was prepared as previously described (8). It contains the following antibiotics (per liter): cefoperazone, 15 mg; rifampin, 10 mg; colistin, 10,000 IU; and amphotericin B, 2 mg. Medium M2 was identical to medium M1, except that cefoperazone was used at a concentration of 30 mg/liter and colistin was omitted. Medium M3 was composed of a blood-free basal medium (CDA) (Oxoid CM 739) containing (per liter) nutrient broth no. 2 (25 g), bacteriological charcoal (4 g), casein hydrolysate (3 g), sodium deoxycholate (1 g), ferrous sulfate (0.25 g), sodium pyruvate (0.25 g), and agar (12 g) as previously described (4); the basal medium was supplemented with 32 mg of cefoperazone per liter (Oxoid SR 125). Medium M4, known as Preston medium (5), contains *Campylobacter* agar base (Oxoid CM 689) supplemented with (per liter) trimethoprim (10 mg), rifampin (10 mg), polymyxin B (5,000 IU), and cycloheximide (100 mg) (Oxoid SR 117). The filtration system originally described by Steele and McDermott (19) was modified as follows. A 47-mm, 0.45-μm-pore Gelman cellulose triacetate membrane filter was placed on CDA medium in small petri dishes (diameter, 50 mm) (Fig. 1).

Specimens. A total of 5,276 stool specimens (81% from children and 19% from adults; 1,232 solid stools and 4,044 diarrheic stools) submitted to our laboratory for routine culturing of enteric pathogens from February to December 1985 were examined. In study 1 (February to July 1985), medium M1 and medium M3 were compared for their isolation rates of *C. jejuni* and *C. coli* from 2,788 stool specimens. In study 2 (September to December 1985), the four selective media were compared for their isolation rates of *C. jejuni* and *C. coli* from 2,488 stool specimens and for their contamination with fecal flora. The filtration system was tested in parallel with the four media on the last 986 stool specimens (October to December 1985). The aspect of the stools was noted, and the presence of leukocytes and

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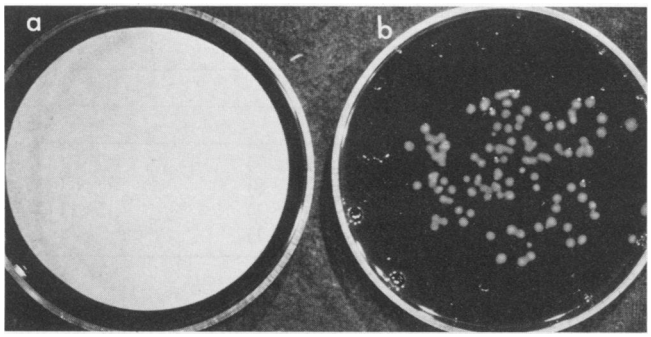

FIG. 1. Filtration method. (a) Application of a membrane filter to blood-free CDA medium in a small petri dish; 6 drops of fecal suspension was placed on the filter. (b) Growth of *Campylobacter* colonies.

erythrocytes in the stools was tested by means of Giemsa staining.

Inoculation. Within 24 h of collection, feces were inoculated directly onto the media; emulsification in saline to obtain a smooth heavy suspension was carried out for solid stools. For the four selective media, the inoculated plates were streaked into four quadrants with a wire loop so that single colonies could be recovered. For the filtration system, 6 drops of fecal suspension was placed on the surface of a filter with a Pasteur pipette. The filter was removed 30 to 60 min after the suspension had been applied.

Incubation. The four selective media were incubated at 42°C, and the small petri dishes with CDA medium were incubated at 37°C; all incubations were done in a special incubator from which two-thirds of the air had been evacuated and replaced by a final gas mixture of approximately 6% oxygen, 6% carbon dioxide, and 88% nitrogen. The selective plates were examined for *C. jejuni* and *C. coli* growth after 24 and 48 h. Results were reported after 48 h. The CDA plates were examined after 3 to 4 days. Suspected *Campylobacter* colonies were stained with a crystal violet stain.

Identification and biotyping. Oxidase, catalase, and nalidixic acid (30 μg) susceptibility tests were done. All isolates were biotyped by the Lior biotyping scheme (13) on the basis of the hippurate hydrolysis test (10), the H_2S test (18), and the DNase test (13).

MICs for isolates. The MICs (milligrams per liter) of the antibiotics present in the selective media, i.e., cefoperazone (Pfizer Inc.), rifampin (Dow-Lepetit Chemical Co.), colistin (Bellon Laboratories), polymyxin B (Pfizer), vancomycin (Eli Lilly & Co.), and trimethoprim (Roche Diagnostics, Div. Hoffmann-La Roche Inc.), were determined for all the *Campylobacter* strains.

The bacterial inoculum was prepared as previously described (20). MICs were determined in liquid medium by using a model 2000 dispenser and inoculator (Dynatech Laboratories, Inc.) (9). *Escherichia coli* ATCC 25922 was simultaneously tested to ensure the potency of each drug. MICs were read after incubation at 42°C for 24 to 48 h in the appropriate gas mixture and were recorded as the lowest concentration of antibiotic inhibiting visible growth.

RESULTS

Study 1 with M1 and M3 media showed similar isolation rates for *C. jejuni* and *C. coli* (i.e., 7.0%). However, three *C. coli* strains were isolated on medium M3 only; these strains were found to be susceptible to colistin (MIC, 0.097 to 0.78 mg/liter). We then decided to carry out a more detailed second study.

During study 2 (September to December 1985), a total number of 128 strains were isolated from 126 different patients (in 2 patients, two different *Campylobacter* strains were isolated, as demonstrated by biotyping and susceptibility testing). These 128 strains were isolated from solid stools in 25 patients and from diarrheic stools in 103 patients (49 patients had bloody diarrhea, and the remaining 54 patients had liquid diarrhea without leukocytes or erythrocytes in their stools). Of these 128 *Campylobacter* strains, 22 strains, including 4 strains isolated with the membrane filter system only (see below), failed to grow upon subculturing. Table 1 shows the frequency of isolation of *C. jejuni* and *C. coli* on the four selective media. Whenever *Campylobacter* spp. were isolated, growth of 69.2, 70.0, 56.6, and 39.1% of the isolates was "pure" on M1, M2, M3, and M4 media, respectively. The frequency of contaminating fecal flora on the four selective media for all 2,488 stool specimens is shown in Table 2.

The membrane filter method with nonselective, blood-free CDA medium was tested simultaneously with the four selective media on the last 986 stool specimens: 52 *Campylobacter* strains were isolated on all the media; 8 strains were isolated by the filtration method only, but 13 strains were not isolated by this method (Table 3). Of the eight *Campylobacter* strains isolated by the membrane filter method only, four strains failed to grow upon subculturing, three strains belonged to *C. jejuni* biotype II, and one strain was a catalase-negative campylobacter with the following characteristics: growth at 37 and 42°C but not at 25°C; hippurate hydrolysis, H_2S, and DNase negative; nalidixic acid susceptible; and trimethylamine *N*-oxide (1) negative.

Table 4 shows the MICs of colistin, polymyxin B, cefoperazone, rifampin, and vancomycin for 106 *Campylobacter* spp. isolated on the selective media as well as by the membrane filter method. (Trimethoprim is not included in Table 4 because all the MICs were ≥100 mg/liter.)

DISCUSSION

The first comparative study between M1 and M3 media, carried out on 2,788 stool specimens from February to July 1985, revealed similar isolation rates for *C. jejuni* and *C. coli*. However, two *C. coli* biotype I strains and 1 *C. coli* biotype II strain were only isolated from medium M3. It was found that the MIC of colistin for both *C. coli* biotype I strains was 0.097 mg/liter, and that for the *C. coli* biotype II strain was 0.78 mg/liter. Therefore, we decided to carry out a more detailed study with four selective media. Medium M2 was

TABLE 1. Isolation rates of *Campylobacter* strains from M1, M2, M3, and M4 media[a]

Medium	No. of strains isolated		No. of strains which failed to grow upon subculturing
	C. jejuni	*C. coli*	
M1	81	14	7
M2	80	14	12
M3	82	11	6
M4	67	10	3

[a] A total of 2,488 stool specimens were tested from September to December 1985, from which 120 campylobacters were isolated by all four selective media. The total percentages of strains isolated from M1, M2, M3, and M4 were 85.0, 88.3, 82.5, and 66.6, respectively. The total percentages of *C. jejuni* and *C. coli* isolated on all four media were 75.0 and 13.3, respectively. A total of 11.6% of strains failed to grow upon subculturing.

TABLE 2. Selectivity of M1, M2, M3, and M4 media in inhibiting the growth of contaminating fecal flora[a]

Medium	No. (%) of plates with:				
	No contaminants	Contaminants in quadrant:			
		1	2	3	4
M1	1,627 (65.4)	523 (21.0)	195 (7.8)	66 (2.7)	77 (3.1)
M2	1,758 (70.7)	415 (16.7)	162 (6.6)	70 (2.8)	83 (3.3)
M3	1,553 (62.4)	511 (20.5)	162 (6.6)	126 (5.1)	137 (5.5)
M4	1,003 (40.3)	981 (39.4)	298 (12.0)	128 (5.1)	78 (3.1)

[a] A total of 2,488 stool specimens were tested.

derived from medium M1, except that colistin was omitted and, since we were expecting more growth of contaminating fecal flora, we decided to raise the concentration of cefoperazone to 30 mg/liter. To evaluate a possible inhibition of *Campylobacter* strains by cefoperazone, we also included a cephalosporin-free medium, medium M4. However, during study 2, Ng and colleagues published a paper (15) suggesting that some *Campylobacter* strains may be somewhat susceptible to antibiotics present in the selective media. We therefore decided to use an antibiotic-, blood-free CDA medium (4), for the isolation of *Campylobacter* strains from stool specimens by the filtration method of Steele and McDermott (19). Finally, the comparison between M1 and M2 media was carried out blindly, because both media look identical; unfortunately, the same approach was not possible for medium M3 (black) and medium M4 (lysed blood).

Similar isolation rates were again found for M1 and M3 media but medium M2 showed the highest isolation rate. However, there was no statistically significant difference among the isolation rates of these three selective media. Medium M4 was found to be very unsatisfactory for the primary isolation of *Campylobacter* spp. Furthermore, the difference in the isolation rate of *Campylobacter* spp. among medium M4 and the other three media was found to be statistically significant ($P < 0.05$ being regarded as significant with the chi-square test). Our findings confirm the finding of Bolton and colleagues (4) that a charcoal-based medium is as effective as blood-containing ones for growing *Campylobacter* spp. Swarming and effuse colonies were found on this medium as well as on the blood-based M1 and M2 media. The distinction between *Campylobacter* colonies and colonies of contaminating fecal organisms was less obvious on medium M4, obliging us to carry out staining of suspected *Campylobacter* colonies on this medium more frequently; thus, we found medium M4 to be more time-consuming than the other media.

The appearance of atypical *Campylobacter* colonies and the low selectivity of medium M4 may explain its low isolation rate. Indeed, this medium was found to be far less selective than M1, M2, and M3 media. Suppression of contaminating fecal flora was lower on medium M3 than on M1 and M2 media, medium M2 being the most selective medium. Indeed, blood-free selective medium M3 provided less suppression of gram-positive organisms and yeasts than did M1 and M2 media, explaining the higher number of plates with medium M3 that showed contamination in the fourth quadrant (Table 2). These findings, together with the findings of Karmali and colleagues (11), strongly suggest the need for the incorporation in selective media of drugs against not only gram-negative organisms (cefoperazone) but also gram-positive organisms (rifampin and vancomycin) and yeasts (amphotericin B and cycloheximide).

With the filtration system and an antibiotic- and blood-free medium, eight more *Campylobacter* strains were found: four strains failed to grow upon subculturing, three strains belonged to *C. jejuni* biotype II, and 1 strain was a catalase-negative *Campylobacter* strain whose characteristics resem-

TABLE 3. Isolation rates of *Campylobacter* strains from M1, M2, M3, and M4 media and by the filtration method[a]

Medium or method	No. of strains isolated			No. of strains which failed to grow upon subculturing
	C. jejuni	*C. coli*	Catalase-negative *Campylobacter* strain	
M1	34	3	0	1
M2	32	5	0	3
M3	35	4	0	1
M4	31	4	0	0
Filtration	32	2	1	4

[a] A total of 986 stool specimens were tested from October to December 1985, from which 52 campylobacters were isolated by all four selective media and the filtration method; 8 of the 39 *Campylobacter* strains isolated with the filtration system were not recovered from any of the four selective media.

TABLE 4. MICs for 106 *Campylobacter* spp.

Organism (*n*) and drug	MIC (mg/liter)[a]		
	Range	50%	90%
All isolates			
Polymyxin B	≤0.097–50	6.25	12.50
Colistin	≤0.097–>100	6.25	12.50
Rifampin	0.195–>100	50	100
Vancomycin	3.12–>100	>100	>100
Cefoperazone	25–>100	>100	>100
C. jejuni (89)			
Polymyxin B	3.12–50	6.25	12.50
Colistin	1.56–>100	6.25	12.50
Rifampin	12.50–>100	50	100
Vancomycin	3.12–>100	>100	>100
Cefoperazone	25–>100	>100	>100
C. coli (16)			
Polymyxin B	0.78–6.25	3.12	6.25
Colistin	0.195–6.25	1.56	3.12
Rifampin	0.195–100	25	100
Vancomycin	6.25–>100	>100	>100
Cefoperazone	25–>100	>100	>100
Coagulase-negative *Campylobacter* strain (1)			
Polymyxin B	≤0.097		
Colistin	≤0.097		
Rifampin	6.25		
Vancomycin	100		
Cefoperazone	25		

[a] 50 and 90%, MIC for 50 and 90% of isolates, respectively.

bled those of the catalase-negative or -weak-positive *Campylobacter* group (16). Steele and McDermott (19) also noticed that a number of *Campylobacter* strains isolated by the filtration system failed to grow upon subculturing. Of the four strains which failed to grow, two were isolated from patients with bloody diarrhea, one was associated with watery diarrhea, and one was isolated from solid stools. The catalase-negative *Campylobacter* strain was isolated from a patient with watery diarrhea. On the other hand, 13 *Campylobacter* spp. could only be isolated with the selective media, a result which was probably due to the low sensitivity of the filtration method. Indeed, a negative culture with the filtration method was usually associated with limited growth on the selective media, indicating that the filtration method may not detect small numbers of campylobacters in stools.

The MICs of six antibiotics used in the selective media were determined. Amphotericin B was excluded because it is added for its fungicidal effect and is not expected to have an effect on bacteria (2). The MICs were read as soon as adequate growth was observed in control plates, because prolonged incubation is not recommended for colistin, polymixin B, and rifampin (21). Table 4 shows the MICs of polymixin B, colistin, rifampin, vancomycin, and cefoperazone (the MICs of trimethoprim were ≥100 mg/liter). Table 4 shows that *C. coli* was more susceptible to antibiotics, particularly polymyxin B and colistin, than was *C. jejuni*, in agreement with the results of Ng and colleagues (15). It was found that the growth of *C. coli* was reduced on medium M1 in comparison with medium M2. However, this reduction did not result in a lower isolation rate of *C. coli* on medium M1 (Table 1), although investigators with little experience may fail to recognize *Campylobacter* colonies if only a few have grown on the primary plate. We were not able to evaluate the effect of polymyxin B, which has a mode of action similar to that of colistin (12), on *Campylobacter* spp. in medium M4, because of the high degree of contamination with fecal flora on these plates and because we had not included an identical medium without polymyxin B. However, none of the *Campylobacter* strains isolated on medium M4 were susceptible to cefoperazone. The high susceptibility of the catalase-negative *Campylobacter* strain explains why this strain could be isolated from the antibiotic-free medium.

In conclusion, this study confirms the promising results of the charcoal-based, blood-free medium of Bolton et al. (4); this medium will be of great value in Third World countries, where supplies of sterile blood are not always available. Unfortunately, antibiotics are still required to make it selective. The difference in the isolation rate among M1, M2, and M3 media was not statistically significant. However, because of the inhibitory effect of colistin and polymyxin B on *Campylobacter* spp., particularly *C. coli* but also *Campylobacter*-like organisms (14), we feel that these drugs should no longer be included in selective supplements. With blood-free medium M3, contamination with gram-positive organisms and yeasts may occur. Therefore, we recommend medium M2, i.e., cefoperazone at 30 mg/liter, rifampin at 10 mg/liter, and amphotericin B at 2 mg/liter; however, the combination of Karmali and colleagues (11), i.e., cefoperazone at 32 mg/liter, vancomycin at 20 mg/liter, and cycloheximide at 100 mg/liter, is probably also suitable.

Finally, this study emphasizes the need for an antibiotic-free medium. This type of medium would not only raise the isolation rate of *C. jejuni* and *C. coli* but would also result in the isolation of new *Campylobacter* spp. which seem to be associated with diarrhea. In the meantime, we will continue to use the filtration method also because the use of small petri dishes makes it cheap and easy to perform.

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