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Laboratory and Clinical Evaluation of Isolation Media for *Campylobacter jejuni*

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Six selective isolation media were evaluated for their ability to support the growth of *Campylobacter jejuni*. Colony counts of 70 isolated strains of *C. jejuni* and recovery studies on these strains in simulated positive feces samples demonstrated that Bolton and Hutchinson' charcoal, cefoperazone, deoxycholate agar and Karmali's charcoal-based selective medium produced the highest recovery rates with the greatest suppression of other fecal flora. *C. jejuni* colonies were more easily recognized on charcoal-based selective medium. A clinical evaluation performed on 2,780 human, animal, and avian feces specimens confirmed the results of the laboratory investigation. From human samples, 4 more strains of *C. jejuni* were isolated on charcoal-based selective medium than were isolated on Skirrow medium, and 19 more strains of *C. jejuni* or *C. coli* were isolated on charcoal-based selective medium from animal specimens. Suppression of normal fecal flora was also greater on charcoal-based selective medium.

In recent years *Campylobacter jejuni* has been recognized as a major cause of gastroenteritis in humans. There have been many comparative studies of selective media for the isolation of *C. jejuni* (1, 3–6, 8, 12, 16). Results from these studies vary according to the media compared and the source of specimens tested. It is therefore difficult to determine if any of the published media formulations are truly superior.

The objective of this investigation was to compare six different isolation media for their ability to permit easy recognition and maximum recovery of *C. jejuni* in pure culture and in simulated positive feces. The media evaluated were those described by Skirrow (Skirrow medium) (14), Lauwers et al. (Butzler medium) (10), Blaser et al. (Blaser-Wang medium) (2), and Bolton and Robertson (Preston medium) (6), as well as the charcoal-based selective medium (CSM) described by Karmali et al. (8) and charcoal, cefazolin, sodium deoxycholate agar (CCDA) described by Bolton et al. (5). This last medium was subsequently modified by the substitution of cefoperazone for cefazolin (modified CCDA) (D. N. Hutchinson and F. J. Bolton, Letter to the Editor, J. Clin. Pathol. 37:956–957, 1986). An evaluation of clinical specimens was designed to test the laboratory observations by comparing the most successful medium from this laboratory evaluation with Skirrow medium, which was the medium that was in routine use in the participating laboratories at the time of the trial. The clinical evaluation was also designed to determine if the efficacy of the selective isolation media varied with the source and condition of the specimen.

MATERIALS AND METHODS

Test strains. Seventy clinical isolates of *C. jejuni* were obtained from the Ontario Ministry of Health, Laboratory Services Branch (Toronto Public Health Laboratory), Toronto, Ontario, Canada, and from hospitals in Hamilton, Ontario, Canada. The identification of *C. jejuni* was confirmed by microscopic appearance; catalase, oxidase, and hippurate hydrolysis; growth at 25, 37, and 42°C; resistance to cephalothin; and susceptibility to nalidixic acid (11). All cultures were maintained at −70°C in tryptic soy broth (GIBCO Canada, Burlington, Ontario, Canada) containing 20% (vol/vol) glycerol. When required for use, the strains were subcultured on Columbia agar base (Oxoid Canada Inc., Nepean, Ontario, Canada) containing 5% defibrinated horse blood (Qualicum Scientific, Ottawa, Ontario, Canada) and incubated microaerobically at 42°C for 24 h in sealed jars containing nitrogen, carbon dioxide, and oxygen generated by gas kits for *Campylobacter* culture (Oxoid Canada). Working suspensions were prepared from these cultures in 0.01 M phosphate-buffered saline (PBS; pH 7.3). The cell density was adjusted visually by comparison with a 0.5 McFarland standard. Further dilutions were made from the standardized suspensions by the protocols set out below.

Selective isolation media. The following media were prepared according to the instructions of the authors: Skirrow, Butzler, Blaser-Wang, Preston, modified CCDA, and CSM. Selective supplements for all media with the exception of

TABLE 1. Recovery of 70 *C. jejuni* strains on six selective media

Medium	Colony count[a]	*P* value[b]
Blood agar control	7.95 ± 0.36	
Skirrow	7.82 ± 0.48	NS[c]
Butzler	7.77 ± 0.51	<0.05
Blaser-Wang	7.70 ± 0.56	<0.05
Preston	7.76 ± 0.52	<0.05
Modified CCDA	7.91 ± 0.36	NS
CSM	7.83 ± 0.37	NS

[a] Log_{10} mean colony counts ± standard deviation.
[b] Significance determined by Student's *t* test for unpaired samples.
[c] NS, Not significant.

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TABLE 2. Isolation of *C. jejuni* from 70 simulated positive feces samples

Medium	No. (%) of strains isolated after incubation for:	
	24 h	48 h
Blood agar control	69 (99)	70 (100)
Skirrow	39 (56)	67 (96)
Butzler	38 (54)	60 (86)
Blaser-Wang	17 (24)	31 (41)
Preston	32 (46)	64 (91)
Modified CCDA	61 (87)	69 (99)
CSM	63 (90)	68 (97)

TABLE 4. Isolation of *C. jejuni* or *C. coli* from clinical specimens

Source	No. tested	No. (%) of strains isolated on the following media at the indicated times:			
		Skirrow medium		CSM	
		24 h	48 h	24 h	48 h
Hospital patients	1,680	23 (1.4)	32 (1.9)	22 (1.3)	32 (1.9)
Specimens in transport medium	876	8 (0.9)	46 (5.3)	8 (0.9)	50 (5.7)
Animal specimens	224	45 (20)	60 (27)	66 (30)	79 (35)
Total	2,780	76 (2.7)	138 (5.0)	96 (3.5)	161 (5.6)

CSM were supplied by Oxoid Canada. The selective supplement for CSM was prepared in the laboratory by using assayed antibiotic powders.

Quantitative assessment of media. The various media were tested for their ability to support the growth of *C. jejuni* in pure culture. Prior to use, the media were dried for 1 h at 35°C to inhibit swarming of the organisms and to produce discrete colonies for counting purposes. Five 10-fold dilutions were made in PBS from the standardized suspensions of *C. jejuni*. The last three dilutions were inoculated onto each test medium and a nonselective blood agar control containing Columbia blood agar base and 5% defibrinated horse blood (control medium) by using a Steers replicator (15) with inoculating pins (diameter, 3 mm). Plates were inoculated in triplicate. The inoculated media were incubated microaerobically for 24 h at 42°C.

Statistical analysis of colony counts. The mean colony count for each of the 70 strains of *C. jejuni* was determined for each medium. Log_{10} values were calculated from the means and analyzed by Student's *t* test for unpaired samples. Significant differences were determined at the 0.05 level. All statistical calculations were performed on logarithmic values to stabilize the variance with respect to the mean.

Inoculation of simulated positive specimens. Seventy feces specimens previously found to be negative for pathogenic enteric bacteria by standard methods (13) were emulsified in PBS to obtain homogenous feces suspensions. The standardized suspension of each of the 70 strains of *C. jejuni* previously described was diluted in PBS and added to a feces suspension to obtain a final concentration of 10^5 CFU/ml. This concentration of organisms was found to be the minimum necessary to ensure recovery of an identifiable growth of *C. jejuni* from the feces inoculum. A 50-μl fraction of the feces suspension was inoculated onto each selective isolation medium and the control medium and streaked in a manner designed to give isolated colonies. A growth control was also made by using PBS as the diluent instead of feces, and this was inoculated on the control medium in the same way. All plates were incubated at 42°C and were examined after 24 and 48 h of incubation.

Quantitation of fecal flora was made by comparison of the amount of growth on the selective media with that on the control medium. Results were based on the observation of a 75% reduction in fecal flora compared with that in the control medium. *C. jejuni* was noted as being present or absent. Colonies morphologically resembling *Campylobacter* spp. were identified by the same criteria used for the test strains.

Clinical evaluation. Based on the results of the laboratory evaluation, CSM was selected for the evaluation of clinical specimens. A total of 2,780 human, animal, and avian specimens of feces were tested during a 5-month period. Freshly collected human feces (1,680 specimens) were examined at the Henderson General Hospital and Chedoke-McMaster Hospitals in Hamilton. Human feces (876 specimens), collected into the transport medium described by Cary and Blair (7) and in transit for up to 4 days, were examined at the Toronto Public Health Laboratory. A total of 224 specimens collected from various species of animals and birds were examined at the Veterinary Laboratory Services Branch, Guelph. All specimens were inoculated on Skirrow medium and CSM in random order. The plates were incubated microaerobically at 42°C and examined for *C. jejuni* and normal fecal flora at 24 and 48 h. Colonies morphologically resembling *C. jejuni* were identified by conventional methods. The animal and avian isolates were not differentiated beyond the *C. jejuni* and *C. coli* group.

RESULTS

Laboratory evaluation of media. The mean of three determinations for each of 70 *C. jejuni* strains on each selective

TABLE 3. Suppression of fecal flora from 70 simulated positive fecal samples

Medium	No. (%) of plates with 75% reduction of fecal flora compared with control after incubation for:	
	24 h	48 h
Skirrow	46 (66)	38 (54)
Butzler	56 (80)	47 (67)
Blaser-Wang	28 (40)	15 (21)
Preston	58 (83)	50 (71)
Modified CCDA	64 (91)	59 (84)
CSM	64 (91)	59 (84)

TABLE 5. Isolation of *C. jejuni* or *C. coli* from animals and birds

Source	No. of samples tested	No. (%) positive on:	
		Skirrow medium	CSM
Swine	99	29 (29)	48 (48)
Chickens	27	13 (48)	14 (51)
Cattle	23	4 (17)	4 (17)
Mink	13	5 (38)	5 (38)
Dogs	13	1 (8)	1 (8)
Cats	15	0 (0)	0 (0)
Other[a]	34	8 (23)	7 (21)
Total	224	60 (27)	79 (35)

[a] Includes cockatiel, pigeon, fox, cougar, rabbit, parrot, horse, goat, and monkey.

TABLE 6. Suppression of fecal flora from clinical specimens on campylobacter isolation media

Source	No. of specimens tested	No. (%) of specimens with 75% reduction of fecal flora on the following media at the indicatd times:			
		Skirrow medium		CSM	
		24 h	48 h	24 h	48 h
Hospital patients	1,680	943 (56)	601 (36)	1,321 (79)	1,051 (63)
Specimens in transport medium	876	520 (59)	271 (31)	713 (81)	476 (54)
Animal specimens	224	16 (7)	8 (4)	76 (34)	60 (27)
Total	2,780	1,479 (53)	880 (32)	2,110 (76)	1,587 (57)

medium are shown in Table 1. By comparison with the control medium, *C. jejuni* colony counts were reduced on Butzler, Blaser-Wang, and Preston media ($P < 0.05$). Results obtained with other selective media were comparable to those obtained with the control medium.

From simulated positive specimens (Table 2), the isolation of *C. jejuni* was greatest on modified CCDA and CSM after both 24 and 48 h of incubation, with reduced isolation noted on Skirrow, Preston, Butzler, and Blaser-Wang media. Of the 70 strains, 1 failed to grow on the control medium after 24 h, but was recovered after 48 h.

Normal fecal flora grew in all four streaked sections of the control medium from all 70 specimens of feces. There was more suppression of fecal flora after 24 h than after 48 h of incubation on selective media (Table 3). Modified CCDA and CSM showed the greatest suppression of fecal flora. There was less suppression of normal flora demonstrated on Preston, Butzler-Wang, Skirrow, and Blaser media. While modified CCDA and CSM performed almost identically in the initial laboratory evaluation, CSM was selected for comparison with Skirrow medium to test clinical specimens because campylobacter colonies were more easily recognizable on CSM than on modified CCDA. Colonies on both media appeared flat, moist, and semitranslucent, but spread more readily on CSM than on modified CCDA.

Clinical evaluation of media. Results of the isolation of *C. jejuni* or *C. coli* from human and animal specimens on Skirrow medium and CSM are shown in Table 4. The isolation of *C. jejuni* from fresh human feces was identical on both Skirrow medium and CSM. Isolation of *C. jejuni* from human feces that were mailed in to the laboratory was greater on CSM after 48 h of incubation. The isolation of *C. jejuni* or *C. coli* from animal and avian fecal samples was greater on CSM after both 24 and 48 h of incubation (Table 5). CSM was far superior to Skirrow medium in its ability to suppress the growth of normal flora after both 24 and 48 h of incubation in all specimens tested (Table 6).

DISCUSSION

The conclusion drawn from most evaluations performed on selective media for the isolation of *C. jejuni* or *C. coli* is that optimal recovery requires the use of more than one selective medium. This is not always possible in a busy routine diagnostic laboratory. The purpose of this evaluation was to determine which single medium performed most satisfactorily when tested under different conditions.

The ability to support the growth of *C. jejuni* and inhibit normal flora are the most important features of a selective isolation medium. The newer formulations of media, modified CCDA and CSM, which include cefoperazone as a selective agent, are most effective for this purpose, as shown in Tables 2 and 3. Cefoperazone is a broad-spectrum cephalosporin with enhanced activity against pseudomonads and members of the family *Enterobacteriaceae*, which accounts to a large extent for its powerful effect as a selective agent. This feature and the isolation rates of *Campylobacter* spp. achieved with CSM made it the recommended medium.

Results observed with modified CCDA were similar to those observed with CSM, but CSM produced the most typical, easily recognizable colonial morphology of the strains tested.

Results observed with Blaser-Wang medium were disappointing. The colonial morphology of *C. jejuni* was atypical on this medium, with many strains being indistinguishable from coliforms. Only 21% of the simulated specimens gave 75% reduction in normal fecal flora compared with 84% of those same specimens grown on both CSM and modified CCDA.

The occurrence of cephalosporin-susceptible strains of *C. coli* has been reported (9). This observation could raise concern about the routine use of CSM since it contains cefoperazone. It should be noted that *C. coli* was not isolated from any human samples in this study. However, data shown in Table 5 indicate that 19 (8.0%) more *C. jejuni* or *C. coli* were isolated from animal sources on CSM than on Skirrow medium (which does not contain a cephalosporin), and the majority of these isolates were from swine. CSM was 19% more effective than Skirrow medium for the isolation of *C. jejuni* or *C. coli* from swine. A subsequent investigation proved that all swine isolates from CSM were in fact *C. coli*. These data suggest that *C. coli* can effectively be isolated with CSM.

The results of this evaluation show that more recently formulated campylobacter isolation media such as CSM or modified CCDA that contain cefoperazone and charcoal are superior to earlier formulations. Not only is the recognition of campylobacters greatly enhanced, but the greater suppression of normal flora significantly reduces the time that is spent on screening procedures. Results of this study also illustrate the importance of including simulated samples and clinical specimens in addition to pure cultures in the evaluation of media performance.

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