JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1988, p. 518–523
0095-1137/88/030518-06$02.00/0

Vol. 26, No. 3

Characterization of Freshly Isolated *Campylobacter coli* Strains and Suitability of Selective Media for Their Growth

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Typical and atypical *Campylobacter* strains were isolated from the colons of cattle and swine by techniques that enabled the selective pressures of antibiotics to be avoided. Some cephalothin-susceptible strains and a strain with an indeterminate hippurate reaction were classified as *Campylobacter coli* by DNA homology testing. Tetracycline-resistant isolates were obtained from animals with no recorded exposure to antibiotics. A selection of 12 *C. coli* and 6 *C. jejuni* strains was used to determine the ability of fresh isolates to grow on a range of selective media. *C. coli* isolates were inhibited more than *C. jejuni* on selective media containing antibiotics. The least inhibitory media were Skirrow medium (M. B. Skirrow, Br. Med. J. 2:9–11, 1977) and the charcoal-based media developed by Hutchinson and Bolton (D. N. Hutchinson and F. J. Bolton, J. Clin. Pathol. 37:956–957, 1984) and Karmali et al. (M. A. Karmali, A. E. Simon, M. Roscoe, P. C. Fleming, S. S. Smith, and J. Lane, J. Clin. Microbiol. 23:456–459, 1986). The plasmid contents of the isolates did not appear to be related to their sensitivity to growth on selective (antibiotic-containing) media. The study indicates that selective media used to detect *Campylobacter* spp. could select against the isolation of *C. coli*.

Campylobacter coli is reported at a much lower frequency in outbreaks of human gastroenteritis than is *C. jejuni* (14). One possible explanation for this is that the development of selective media for the isolation of *Campylobacter* spp. from clinical specimens and food samples was generally based on the antibiotic resistance of *C. jejuni* or *C. jejuni-C. coli*. *C. coli* and *C. jejuni* are closely related, being distinguished by the hippurate hydrolysis test (10). In an earlier study (23), we observed that *C. coli* is more susceptible than *C. jejuni* to antibiotics used in selective media. However. this could be a laboratory phenomenon, not typical of fresh isolates.

After prolonged subculturing on laboratory media, the drug resistance patterns of cultures may change. Most of these changes are due to the loss of drug resistance plasmids, which can occur when the selective pressure is removed from the culture medium (6). Spontaneous chromosomal mutations to antibiotic resistance have also been observed. Mutational changes in *C. jejuni* and *C. coli* that result in a change from nalidixic acid-susceptible to resistant strains have been reported (29). This phenomenon has also been observed for other DNA gyrase inhibitors, such as enoxacin (29). Therefore, it is reasonable to assume that the antibiotic susceptibilities of laboratory strains of bacteria may differ from those of fresh isolates.

In this study, fresh isolates of *C. jejuni* and *C. coli* were obtained from colons of cattle and swine by plating onto an antibiotic-containing medium and by a filtration technique that does not involve the use of antibiotics in the selective process. The fresh isolates were identified by their phenotypic characteristics and, if necessary, by DNA homology. The antibiotic susceptibilities of the fresh isolates of *C. coli* and *C. jejuni* were determined, especially to antibiotics used in selective growth media. The objective of the study was to determine the ability of fresh isolates of *C. coli* to grow on a range of media in use for the selective culture of *Campylobacter* spp.

MATERIALS AND METHODS

Reference cultures. Reference *Campylobacter* strains used for identification, biotyping, and DNA homology studies included *C. coli* NCTC 11353, *C. jejuni* NCTC 11392, *C. jejuni* NCTC 11168, *C. fetus* subsp. *fetus* ATCC 27374, *C. laridis* NCTC 11352, *C. hyointestinalis* (supplied by J. H. Bryner, National Animal Disease Center, Ames, Iowa), and *C. pylori* (supplied by H. Lior, Laboratory Centre for Disease Control, Ottawa, Canada). Reference strains for MIC determinations included *C. coli* NCTC 11353, *C. jejuni* NCTC 11168, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* Oxford. Laboratory strains *C. jejuni* UA1 and UA526 and *C. coli* UA421 and UA530 were included for cross-reference with our previous study (23).

All of the *Campylobacter* reference strains were stored at −70°C in a sterile preservation medium containing glycerol (40%) and trisodium citrate (1.8%). *Campylobacter* cultures were subcultured twice on Mueller-Hinton (MH) agar (Oxoid Ltd., Nepean, Ontario, Canada) and incubated for 24 h at 37°C in an atmosphere containing 7% CO_2. Other species were subcultured in a similar manner on nutrient agar and incubated aerobically at 37°C.

Sources and isolation of *Campylobacter* spp. Sections of the spiral colons of cattle and swine were obtained at the time of slaughter from a local slaughterhouse and the Agriculture Canada Research Station, Lacombe, Alberta, Canada. Animals raised at the Lacombe Research Station had a known antibiotic history. Only trimethoprim had been used to treat cases of scours during weaning; otherwise, antibiotics had not been used for therapeutic or growth purposes. In the laboratory, the gut contents were removed without the inner linings being scraped. Approximately 15 cm of semicleaned colon was placed in a plastic bag with 50 ml of sterile 0.85% saline and mixed for 15 s in a Stomacher blender (model 400; Colworth, London, England).

Campylobacter spp. were isolated either by streaking

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unfiltered suspensions onto MH agar and MH agar containing polymyxin (2,500 IU/liter), trimethoprim (5 mg/liter), and vancomycin (10 mg/liter) (SK medium [27]) or by streaking the suspension filtrate (pore size, 0.65 μm; Millipore Corp., Mississauga, Ontario, Canada) onto MH and SK media. All plates were incubated at 42°C in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2 for 24 or 48 h. Typical *Campylobacter* colonies were selected for examination by phase-contrast microscopy, and colonies with small, spiral cells demonstrating a darting motility were streaked onto MH agar. Isolated colonies were subcultured onto MH plates. Cells that grew on the MH plates were suspended in glycerol-citrate medium and stored at −70°C.

Selection of isolates. Up to 25 typical *Campylobacter* colonies were selected from MH plates, and 1 was selected from SK plates. The isolates were inoculated aseptically onto MH plates containing tetracycline (8 μg/ml), cephalothin (64 μg/ml), or kanamycin (8 μg/ml) and also onto MH plates containing the antibiotic combinations proposed by Skirrow (27), Wesley et al. (31), Dekeyser et al. (7), and Goossens et al. (9). A representative group of 175 *Campylobacter* cultures was selected on the basis of differences in their antibiotic susceptibility. Antibiotics used in this study were tetracycline, kanamycin, bacitracin, cephalothin, colistin, polymyxin B sulfate, novobiocin, rifampin, trimethoprim, vancomycin (all purchased from Sigma Chemical Co., St. Louis, Mo.), and cefoperazone (Cefobid; Pfizer Canada Inc., Kirkland, Quebec, Canada). Cycloheximide and amphotericin B, used as antifungal agents in some selective media, were also purchased from Sigma.

Identification of isolates. The selected isolates were screened for production of oxidase and catalase and identified by the following tests: growth at 25, 37, and 42°C; nitrate reduction; H_2S production in triple sugar iron agar (22); susceptibility to nalidixic acid (40 μg/ml) and cephalothin (64 μg/ml) in MH agar (14); tolerance to 1% glycine, 3.5% NaCl (25), 2,3,5-triphenyltetrazolium chloride (400 μg/ml) in nutrient agar (16); and production of alkaline phosphatase (17) and urease (5).

Base composition determination and DNA homology studies were done for atypical strains. The total genomic DNA from these strains was isolated from the cell suspension by the method described by Marmur (19) and purified with RNase (bovine pancrease; Calbiochem-Behring, La Jolla, Calif.) and pronase (Boehringer Mannheim, Dorval, Quebec, Canada). The DNA was dissolved in 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate [pH 7.0]) to contain 1 to 2 mg of DNA per ml. The base composition of the isolates was determined by measuring the thermal denaturation temperature (T_m [20]) of the DNA in 0.1× SSC with a temperature-programmed spectrophotometer (DU-8; Beckman Instruments, Inc., Fullerton, Calif.). DNA from *Micrococcus lysodeikticus* (Sigma) was used as the reference.

DNA homology was determined by using the slot blot hybridization technique described in our previous study (23a). The degree of homology was determined by visually examining the intensity of the DNA slots on the autoradiograms and by cutting out the nitrocellulose filter strips containing individual slots and determining the radioactivity in a scintillation counter (model LS6800; Beckman).

Characterization of isolates. Isolates were differentiated by the biotyping system proposed by Lior (18), and selected isolates were also differentiated by their plasmid profiles. Plasmid profiles were determined by the method described by Birnboim and Doly (2) for extraction of plasmid DNA, followed by gel electrophoresis in 0.7% agarose in Tris-borate-EDTA buffer at 60 V. *E. coli* plasmids of known molecular size (in kilobases) were used as standards, including the following: pUC8, 2.7; pBR322, 4.0; RSF1030, 8.5; S-a, 35.4; R4776, 50.8; RP4, 58.5; RIP69, 72.4; LT_2, 92.4; RA-1, 132.4; R40-a, 147.8; R27, 172.5; Rts-1, 194.0; and R478, 255.6. *C. jejuni* UA466 and UA650 (28) were included as positive and negative controls, respectively. The MICs for 175 isolates were determined on MH plates with twofold serial dilutions of antibiotics, as described previously (23).

Growth of fresh isolates on selective media. The selective media used in this study are listed in Table 1. Each medium was prepared by the procedure described by the authors. The plates were dried overnight at room temperature in the dark. Medium M5, developed by Lauwers et al. (15), was prepared with and without 10% added sheep blood. MH agar with no additions and MH agar with added blood were used as reference media.

On the basis of an earlier study (23), a replicator method was developed for comparative growth of *C. coli* and *C. jejuni* on the selective media. The 18 selected fresh isolates and 4 reference strains from our previous study (23) were grown in MH broth (Oxoid) at 37°C for 24 h in an atmosphere containing 7% CO_2. Serial dilutions were prepared with sterile 0.85% saline solution to give a range of dilutions from 10^{-1} to 10^{-6}. A 0.3-ml sample of each dilution, including the undiluted 24-h culture, was transferred to a well in a Steers replicator. The plates inoculated by using the Steers replicator were dried in a sterile laminar flow hood. The inoculated plates were incubated at 42°C in jars containing an atmosphere of 5% CO_2, 10% O_2, and 85% H_2. Each jar contained an open dish with 3 ml of glycerol on blotting paper to prevent condensation of moisture on the surface of the agar. After 20 and 40 h of incubation the plates were examined under a stereomicroscope, and spots containing 15 to 200 colonies were counted. The experiment was done in triplicate.

RESULTS

Campylobacter spp. were isolated from 43% of 40 cattle colons and 92% of 52 swine colons. The isolation rate from cattle colons was 14 of 40 (35%) by the filtration method and 12 of 40 (30%) without filtration; similarly, for the swine colons the isolation rate was 44 of 52 (85%) by the filtration method and 40 of 52 (77%) without filtration. A total of 108 isolates was selected by the filtration method, of which 40 were from cattle and 68 were from swine. By using the selective medium (SK), a total of 67 isolates was selected; 19 from cattle and 48 from swine.

Identification and characterization of isolates. All of the 59 isolates from cattle colons were *C. jejuni*. Of 103 isolates from swine colons, 100 (97%) were identified as *C. coli*. All isolates were oxidase and catalase positive and grew at 37 and 42°C. Only three isolates from swine colons grew at 25°C. One of these isolates grew better at 37°C than at 42°C, and its biochemical characteristics suggested that it was most probably a strain of *C. fetus* subsp. *venerealis*. The other two isolates that grew at 25°C had phenotypic characteristics similar to those of *C. coli*. Subsequently, they were confirmed as *C. coli* on the basis of G+C content and DNA homology.

A total of 10 nalidixic acid-resistant (Nal^r) strains was isolated, 8 of which could not be characterized at the species level. Some isolates were susceptible to cephalothin at 16 to 64 μg/ml. Eight of the cephalothin-susceptible (Cep^s) strains were also susceptible to nalidixic acid. They were identified

TABLE 1. Selective media used for quantitative comparisons of the growth of *C. coli* and *C. jejuni*

Medium	Antibiotics	Concn (mg/liter or IU/liter)[a]	Reference
M1	Vancomycin	10	27
	Polymyxin B	2,500	
	Trimethoprim	5	
M2	Vancomycin	10	12
	Polymyxin B	5,000	
	Trimethoprim	5	
M3	Vancomycin	10	3
	Polymyxin B	2,500	
	Trimethoprim	5	
	Cephalothin	15	
	Amphotericin B	2	
M4	Polymyxin B	10,000	7
	Bacitracin	25,000	
	Novobiocin	5	
	Actidione	50	
M5	Colistin	10,000	15
	Bacitracin	25,000	
	Cephalothin	15	
	Novobiocin	5	
	Actidione	50	
M6	Polymyxin B	20,000	31
	Rifampin	25	
	Cefsulodin	6.25	
M7	Colistin	10,000	9
	Cefoperazone	15	
	Rifampin	10	
	Amphotericin B	2	
M8	Colistin	10,000	26
	Cephalothin	15	
	Amphotericin B	1	
M9	Colistin	40,000	24
	Cephalothin	15	
	Bacitracin	25,000	
	Novobiocin	5	
	Actidione	50	
M10	Cefoperazone	32	13
	Vancomycin	20	
	Actidione	100	
M11	Cefoperazone	32	11
M12	Polymyxin B	2,500	30
	Trimethoprim	5	
	Rifampin	10	
	Actidione	100	

[a] Polymyxin B and colistin are expressed in IU per liter; other antibiotics are expressed in milligrams per liter.

as *C. coli* biotype I or *C. jejuni* biotype II. Bacteria with different phenotypes were isolated from the same colon; for example, *C. jejuni* biotypes I and II were isolated from one cattle colon. Different *Campylobacter* spp. were also isolated from the same colon; for example, one swine colon contained typical and atypical *C. coli*, as well as other unidentifiable *Campylobacter*-like organisms.

Typical and atypical *C. coli* and *C. jejuni* isolates had G+C contents within the range of 29 to 33 mol%. Atypical *Campylobacter* strains included the Cep^s isolates, the strain with an indeterminate hippurate reaction, and the strain that grew at 25°C. Isolates that were unclassifiable from their phenotypic characteristics had a relatively high G+C content (37 to 42 mol%). Most *C. coli* and *C. jejuni* isolates were biotype I. Only one *C. jejuni* isolate was obtained from swine, and *C. coli* was not isolated from cattle. A total of 23 tetracycline-resistant (Tc^r) isolates was obtained, 12 from four animals with no previous exposure to antibiotics. Kanamycin-resistant isolates were not detected.

Plasmid profiles of selected strains were used to aid in strain differentiation. *C. jejuni* and *C. coli* strains with and without plasmids were found. Of 25 selected strains of *C. jejuni* examined, 15 (including nine Tc^r strains) contained one plasmid about the same size (45 kilobases) as UA466 (28). In contrast, a range of plasmid profiles was encountered for 30 selected *C. coli* strains. Besides plasmid-free strains, the *C. coli* isolates contained one to seven plasmids of sizes ranging from 2 to 170 kilobases. Comparing the plasmid profiles of strains isolated from the same colon, we observed that *C. coli* strains with different plasmid profiles were isolated from the same colon.

MIC determinations. MIC results for the eight antibiotics used in selective media for *Campylobacter* spp. are summarized in Table 2. MICs of bacitracin, trimethoprim, and vancomycin were similar for all of the strains tested. All strains tested were resistant to the levels of these antibiotics used in selective media. A greater range in susceptibility to other antibiotics was noted for both *C. jejuni* and *C. coli*. Strains for which cephalothin MICs were between 32 and 64 μg/ml were tested for susceptibility to cefoperazone. For all of them, cefoperazone MICs were >128 μg/ml. In contrast, for Cep^s *C. coli* UA421 from our previous study (23) and a Cep^s derivative of a *C. jejuni* strain from this study, cephalothin and cefoperazone MICs were 4 and 32 μg/ml, respectively. These strains were susceptible to the levels of cefoperazone used in media M7, M10, and M11. On the basis of the MIC data, six pairs of *C. coli* strains (six strains from the selective [SK] isolation method and six corresponding isolates from the filtration technique) and three pairs of *C. jejuni* strains were selected for further study.

Effect of antibiotic combinations. A preliminary study was done to compare counts obtained by a standard plate count method (23) with those obtained by using the replicator method. Assuming that the 3-mm-diameter pin of the Steers replicator delivers 5 μl of inoculum onto the surface of the medium, the count obtained by this method is 50% (average counts of two replicates of four cultures) of that determined by the plate count method. Results for the growth of laboratory strains of *C. coli* (UA421 and UA530) and *C. jejuni* (UA1 and UA526) correlated with the results obtained in our previous study. For example, the Cep^s *C. coli* UA421 did not grow on most of the selective media tested in either study, whereas *C. jejuni* UA1 grew well on most of the media. There was no difference between the counts on MH with and without blood.

The resistance of the selected *Campylobacter* strains to the combinations of antibiotics used in selective media was compared. The number of colonies calculated as CFU per milliliter growing on MH agar was compared with the number of colonies growing on selective media listed in Table 1. The differences in the calculated $\log_{10}$ CFU per milliliter on MH agar and each selective medium are shown in Table 3. The *C. jejuni* strains grew well on all of the selective media, except for the laboratory strains (UA1 and

TABLE 2. Selective-medium antibiotic MICs for 101 strains of *C. coli* and 60 strains of *C. jejuni*

Antibiotic	MIC (μg/ml)[a] for *C. coli*			MIC (μg/ml)[a] for *C. jejuni*		
	Range	50%	90%	Range	50%	90%
Bacitracin[b]	256–>512	>512	>512	256–>512	512	>512
Cephalothin	32–>256	256	256	64–128	128	128
Colistin[c]	<1–16	2	4	1–16	4	8
Novobiocin	8–128	16	64	16–128	32	32
Polymyxin B[d]	<1–8	<1	2	<1–8	<1	4
Rifampin	16–>128	128	128	4–128	16	32
Trimethoprim	256–>256	>256	>256	256–>256	256	>256
Vancomycin	128–>128	>128	>128	128–>128	>128	>128

[a] 50% and 90%, MIC for 50 and 90% of the strains, respectively.
[b] 1 μg = 65.2 IU of bacitracin (Sigma).
[c] 1 μg = 13.6 IU of colistin (Sigma).
[d] 1 μg = 8 IU of polymyxin B sulfate (Sigma).

UA526) on medium M5 without blood. The addition of blood to medium M5 eliminated the inhibitory effect. Medium M9 was inhibitory for the growth of *C. jejuni* UA526 only.

C. coli strains generally grew well on media M1 and M10, and only the cephalothin-susceptible strain (UA421) was inhibited by medium M11. Growth of this strain was inhibited by most media. Medium M6 was the most inhibitory to the *C. coli* isolates: counts for 10 of the 12 strains tested were reduced by at least 5 log cycles. Medium M5 inhibited all *C. coli* strains tested. With the addition of blood to the selective media, the inhibitory effect against some strains was reduced. Only *C. coli* 5a and 5b were not markedly inhibited by medium M9. When the inhibitory effects of the selective media on the isolates obtained by the filtration or selective medium method were compared, no difference in the growth of the isolates obtained by the two methods was observed (Table 3).

The effects of selective media on the growth of *C. coli* and *C. jejuni* did not appear to be related to the presence of plasmids. For example, both *C. coli* 1a and 1b contained a plasmid encoding Tc^r. The growth of *C. coli* 1a and 1b on the selective media (M1 to M11) was similar (Table 3). The Tc^r *C. jejuni* 7a and 7b contained a single plasmid of similar size to that in the Tc^r *C. coli* 1a and 1b. However, the growth of *C. jejuni* 7a and 7b on selective media (M1 to M11) was different from that of *C. coli* 1a and 1b (Table 3). Therefore, the presence of the Tc^r plasmid did not correlate with the growth of *C. coli* and *C. jejuni* on selective media. *C. coli* 2a is a plasmid-free strain, but *C. coli* 2b from the same animal contained two plasmids of 25.7 and 53 kilobases. The growth of these two *C. coli* strains on the selective media was similar (Table 3). The growth of *C. coli* 2a and 2b on the selective media was similar (Table 3); it was also similar to that of *C. coli* 1a and 1b on the selective media (M1 to M11).

TABLE 3. Difference in recovery of *C. coli* and *C. jejuni* on selective media and nonselective MH agar

Strain[a]	Difference in $\log_{10}$ CFU/ml[b] for:												
	M1	M2	M3	M4	M5	M5B[c]	M6	M7	M8	M9	M10	M11	M12
C. coli													
1a	<1	1	1	4	1	<1	≥5	2	<1	4	<1	<1	1
1b	<1	1	1	5	1	<1	≥5	2	<1	4	<1	<1	3
2a	<1	<1	<1	4	1	<1	5	1	<1	4	<1	<1	1
2b	<1	<1	<1	4	1	<1	5	1	<1	4	<1	<1	<1
3a	<1	1	<1	≥5	1	<1	5	4	<1	≥5	<1	<1	5
3b	<1	2	2	≥5	2	<1	5	1	<1	≥5	<1	<1	3
4a	<1	<1	<1	3	1	<1	1	<1	<1	3	<1	<1	1
4b	<1	<1	<1	3	1	1	1	<1	<1	3	<1	<1	1
5a	<1	<1	<1	4	2	<1	4	<1	<1	<1	<1	<1	1
5b	<1	<1	<1	4	2	<1	5	<1	<1	<1	<1	<1	<1
6a	1	3	<1	6	4	4	6	4	3	6	1	<1	5
6b	<1	2	1	5	4	2	6	3	2	5	1	<1	3
UA421	<1	<1	5	≥5	≥5	≥5	≥5	≥5	≥5	≥5	<1	≥5	4
UA530	<1	<1	<1	<1	5	<1	<1	<1	<1	<1	<1	2	3
C. jejuni													
7a	<1	<1	<1	<1	1	<1	1	<1	<1	ND[d]	<1	1	<1
7b	<1	<1	<1	<1	<1	<1	<1	<1	<1	ND	<1	1	1
8a	1	<1	<1	1	<1	<1	<1	1	<1	ND	<1	<1	<1
8b	1	<1	1	<1	1	<1	1	1	<1	ND	<1	1	<1
9a	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
9b	<1	<1	<1	<1	1	<1	<1	<1	<1	<1	<1	<1	<1
UA1	<1	<1	<1	<1	5	<1	<1	<1	<1	<1	<1	<1	<1
UA526	<1	<1	<1	2	5	<1	1	<1	<1	4	<1	<1	1

[a] Strains denoted a and b were isolated from SK medium and by the filtration technique, respectively.
[b] $\log_{10}$ count on MH agar minus $\log_{10}$ count on specific selective medium specified in Table 1 (data corrected to the nearest $\log_{10}$).
[c] M5B refers to medium M5 containing blood.
[d] ND, Not done.

DISCUSSION

The surfaces of the spiral colons of cattle and swine served as a good source of *Campylobacter* strains. This study showed that both the filtration and SK medium methods were equally good for isolating *Campylobacter* organisms. However, this might not be the case if other, more inhibitory selective media were used to isolate the organisms.

In this study, 8 of 175 *C. coli* and *C. jejuni* strains were cephalothin susceptible (MICs, 32 to 64 μg/ml). Cephalothin-susceptible strains have previously been reported (4, 14, 21). Therefore, the reliability of cephalothin resistance as a differential characteristic should be reevaluated.

At present, only a limited number of phenotypic characteristics are available for the identification and taxonomic classification of *Campylobacter* species. The differentiation of *C. jejuni* and *C. coli* relies solely on the hippurate hydrolysis test (10). Therefore, an indeterminate response in this test makes classification by the conventional biochemical tests impossible. DNA homology proved useful for confirming the identity of the strains with an indeterminate hippurate reaction. A semiquantitative method was used to study the DNA homology of some isolates to confirm their taxonomic classification owing to its reliability (1) and simplicity compared with those of either the S1 nuclease or the hydroxyapatite method.

The G+C contents of some of the unidentifiable isolates obtained in this study were relatively high (37 to 42 mol%) compared with the G+C contents of the reference *C. coli* and *C. jejuni* cultures, as well as published data of 30 to 36 mol% (8, 14). An asparagine-fermenting *Campylobacter* sp. isolated from an anaerobic digester was found to have an unusually high G+C content (41.6 mol%); however, the taxonomic position of these unidentifiable isolates remains uncertain (14).

The MIC results with fresh isolates of *C. coli* paralleled those found for laboratory strains of *C. coli* (23). Some of the fresh isolates of *C. jejuni* were more susceptible to cephalothin (MIC, 64 μg/ml) and rifampin (MIC, 4 μg/ml) than the laboratory strains were (23). This study indicates that some selective media would fail to detect some *Campylobacter* spp., especially *C. coli*. Some selective media were sufficiently inhibitory that they could cause *C. coli* present in relatively large numbers to go undetected.

The replicator method for applying serial dilutions of cultures to the surfaces of growth media, used in this study for the evaluation of the inhibitory effect of selective media for *Campylobacter* strains, was comparable with the plate count method used previously (23). This method is less laborious and more economical than the conventional plate count method.

Medium M1 (27), which contains polymyxin B sulfate, trimethoprim, and vancomycin, and medium M10, which contains cefoperazone (a broad-spectrum cephalosporin), vancomycin, and actidione (13), were the least inhibitory of the selective media for recovery of the *C. coli* and *C. jejuni* strains tested. Medium M11, which contains only cefoperazone (11), inhibited the growth of the Cep^s *C. coli* UA421, although this strain grew well on medium M10. The MIC of cefoperazone for UA421 was 32 μg/ml. Therefore, besides the antibiotics added to M10 and M11, other constituents in these media affect the growth of *C. coli* UA421. Both M10 and M11 contain charcoal and sodium pyruvate, but only M10 contains hemin (11, 13). The basal media used in media M10 and M11 are different. Therefore, hemin and other components in medium M10 may have a protective effect on the Cep^s strains. Substituting cephalothin with cefoperazone may reduce contaminants (11, 13), but may still inhibit the growth of some *Campylobacter* strains.

These data confirm our previous study (23), in which we reported that the most inhibitory *Campylobacter* medium is M6 (31), which contains polymyxin B (20,000 IU/liter), rifampin (25 mg/liter), and cefsulodin (6.25 mg/liter). Medium M5 with blood (15) and medium M9 (24) contain the same antibiotics. Medium M9 contains an even higher concentration of colistin (40,000 IU/liter) than M5 (10,000 IU/liter) does. With this increased level of colistin, 10 of the 12 strains of *C. coli* and 1 of the 4 strains of *C. jejuni* were also inhibited. This might be expected, since polymyxin B and colistin have similar modes of action and inhibitory effects on *Campylobacter* strains.

The use of the selective medium (MH with the antibiotics of Skirrow [27]) for isolation of *Campylobacter* strains did not select for strains with increased resistance to antibiotics. On the basis of the results of this study and our previous study (23), we conclude that the inhibition of fresh isolates of *C. coli* on selective media was similar to that reported for laboratory strains. This may account for the lower isolation rates of *C. coli* than *C. jejuni*, because some of the selective media could be too inhibitory for enumeration or isolation of *C. coli*. This might be even more significant for *Campylobacter* strains in the extraenteral environment, such as water and foods.

ACKNOWLEDGMENTS

L.-K.N. and D.E.T. were supported by an Alberta Heritage Foundation for Medical Research Studentship and Scholarship, respectively.

We thank Daren Tobert for his technical assistance in the isolation of *Campylobacter* strains from animals during his work on an Alberta Heritage Foundation for Medical Research Summer Studentship. We acknowledge the cooperation of the Agriculture Canada Research Station at Lacombe, Alberta, Canada.

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