JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1985, p. 510–514
0095-1137/85/100510-05$02.00/0

Vol. 22, No. 4

Inhibition of *Campylobacter coli* and *Campylobacter jejuni* by Antibiotics Used in Selective Growth Media

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The ability of *Campylobacter coli* and *Campylobacter jejuni* to grow in the presence of antibiotics used in selective growth media was compared. MIC data for *C. coli* indicated that some strains were more susceptible to the antibiotics than were the *C. jejuni* strains tested. A reduction of greater than 1 log cycle in the numbers of cells growing on plates containing antibiotics was considered to be a marked level of inhibition. Only one of nine of the antibiotic combinations studied did not markedly inhibit most of the *C. coli* strains tested. Although one *C. coli* strain was not inhibited by any of the antibiotic combinations, the other six strains were inhibited for up to 7 log cycles. The addition of blood or growth supplements reduced but did not eliminate the inhibitory effect. The inhibition of laboratory strains of *C. coli* on media developed for the isolation of *Campylobacter* spp. indicates that the incidence of *C. coli* may be underestimated.

Campylobacter jejuni and *Campylobacter coli* are now recognized as important agents of gastroenteritis. In 1972 a filtration technique for the isolation of *Campylobacter* spp. from stool samples was described (9). In 1977, Skirrow (25) simplified the isolation procedure by using a solid medium containing three antibiotics (trimethoprim, polymyxin B sulfate, and vancomycin) for the primary isolation of *Campylobacter* spp. Subsequently, other antibiotic combinations were proposed as selective agents for the recovery of *C. jejuni* and *C. coli* from various sources (4, 5, 9, 12, 15, 18, 23, 31). Besides differences in the types and concentrations of antibiotics used, there are also differences in the basal media used with the antibiotics (8), giving rise to the possibility of inconsistent results among laboratories.

There are many reports of antibiograms for *C. jejuni*, including a review by Vanhoof in 1984 (34). The MICs of antibiotics used in many of the selective media have also been reported for *C. jejuni* (1, 9, 13, 14, 20, 22, 28, 35). However, there are far fewer reports of antibiograms for *C. coli*. This is due in part to the lower isolation frequency of this organism from patients (16). However, in the past, *C. jejuni* and *C. coli* were not differentiated and were classified as *Campylobacter fetus* subsp. *jejuni* (16). Information on antibiotic susceptibility for the development of media was based primarily on the antibiotic resistance of *C. jejuni*. Selective media for the isolation of *Campylobacter* spp. may be more inhibitory to *C. coli* than to *C. jejuni*. This inhibitory effect against *C. coli* might be even more significant when media are used for enumeration purposes, as, for example, in foods (27).

In this study, the inhibition of *C. coli* by antibiotics used in media selective for *Campylobacter* spp. was compared with the inhibition of selected strains of *C. jejuni*. This was done by determining the MICs of a range of antibiotics used in selective media as well as by comparing the effects of the various antibiotic combinations used in media selective for *Campylobacter* spp. Antibiotic combinations were evaluated by comparing plate counts on a standard basal medium with and without antibiotics.

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MATERIALS AND METHODS

Cultures. A total of 24 *C. coli* strains were selected from our collection for comparison of their antibiotic resistance with that of 6 *C. jejuni* strains. All of these strains were from human and animal sources. The following reference strains were included from type culture collections: *C. coli* CIP 7077 and CIP 7080 and *C. jejuni* NCTC 11353 and NCTC 11392. In addition, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* (Oxford strain), *Streptococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Campylobacter fetus* subsp. *fetus* ATCC 27374, and *Campylobacter laridis* NCTC 11352 were used as reference strains for MIC determinations. All *Campylobacter* strains were stored at −70°C. They were subcultured on Mueller-Hinton (MH) agar before MIC determinations. Other species were subcultured in a similar manner on nutrient agar.

MIC determinations. A *Campylobacter* colony was selected from a 48-h MH agar plate and inoculated into 2 ml of MH broth. The inoculated broth was incubated overnight at 37°C in an atmosphere containing 7% CO_2. Cultures were diluted with Penassay broth (Difco Laboratories, Detroit, Mich.) to approximately 10^7 CFU/ml, so that 10^4 to 10^5 CFU would be inoculated onto antibiotic plates (2) with a Steers replicator (26). Reference cultures other than the *Campylobacter* reference cultures were grown in nutrient broth at 37°C, diluted, and treated in the same manner as the *Campylobacter* cultures.

Antibiotics included in the MIC determinations were bacitracin, cephalothin, colistin, polymyxin B sulfate, novobiocin, rifampin, trimethoprim, and vancomycin (all supplied by Sigma Chemical Co., St. Louis, Mo.). MICs were determined on MH agar containing twofold increases of antibiotic concentrations. All antibiotic plates were used within 2 days of preparation. Plates containing rifampin and novobiocin were stored in the dark. The MIC was defined as the lowest concentration of antibiotic at which there was complete inhibition of growth.

TABLE 1. Antibiotic composition of selective media used for quantitative comparisons of effects of antibiotics on the growth of *C. coli* and *C. jejuni*

Medium (reference)	Antibiotic concn (per liter of medium)									
	Trimethoprim (mg)	Vancomycin (mg)	Polymyxin B sulfate (IU)	Colistin (IU)	Rifampin (mg)	Cycloheximide (mg)	Cephalothin (mg)	Amphotericin B (mg)	Bacitracin (IU)	Novobiocin (mg)
M1 (25)	5	10	2,500							
M2 (15)	5	10	5,000							
M3 (4)	5	10	2,500				15	2		
M4 (5)	10		5,000		10	100				
M5 (9)			10,000			50			25,000	5
M6 (18)				10,000		50	15		25,000	5
M7 (31)			20,000		25		6.25			
M8 (12)				10,000	10		15	2		
M9 (23)				10,000			15	1		

Effect of antibiotic combinations used in selective media. *Campylobacter* strains were selected from the cultures used in the MIC determinations to provide a range of *C. coli* strains with different antibiotic resistance patterns. In addition, three *C. jejuni* strains were also included as a reference for the *C. coli* results. The strains were incubated in brucella broth at 42°C in anaerobic jars containing a modified atmosphere of 5% O_2–10% CO_2–85% N_2. The 24-h brucella broth cultures were examined by phase-contrast microscopy to ensure that the cells were predominantly in the spiral form as opposed to the coccoid form. Coccus-shaped cells are believed to be a degenerative form of *Campylobacter* cells (8). MH agar was used as the basal medium for this study because of our previous observation that this medium supports the maximum recovery of *Campylobacter* cells in the absence of blood or growth supplements (21). The antibiotic combinations listed in Table 1 were added to MH agar. The 24-h cultures were diluted with 0.85% saline because of observations that *Campylobacter* cells suspended in this diluent for up to 60 min retained their viability when plated onto MH agar. Appropriate dilutions were inoculated in triplicate onto each agar medium and spread over the agar surface with a sterile glass "hockey stick." Plates with 30 to 300 colonies were counted. The differences in counts represented differences in the inhibitory effect of the antibiotic combinations. Inoculated plates were incubated at 42°C for 48 h in a modified atmosphere in plastic bags containing glycerol (27). Plates with no colonies or colonies that were too small to count after 48 h of incubation were reincubated for an additional 48 h and then counted.

Initially, neither blood nor growth supplements were added, except to media M5 and M6. These media were prepared with and without blood because they contained novobiocin, which is known to be bound by serum protein (2). Therefore, the addition of blood may reduce the inhibitory effect of novobiocin in these media. Furthermore, the agar concentration of these media was increased to 2% to prevent swarming of the cells. Subsequently, media that were inhibitory to *C. coli* or *C. jejuni* were retested with growth supplements (31) or sheep or lysed horse blood added at recommended levels (4, 5, 9, 12, 15, 23, 25).

RESULTS

MIC determinations. All cultures grew well on MH agar without antibiotics. MICs for *E. coli*, *S. aureus*, *S. faecalis*, and *P. aeruginosa* were within the published ranges for cephalothin, colistin, polymyxin, trimethoprim, and vancomycin (10, 29) and were consistent between trials. Suspensions of *Campylobacter* cells prepared from colonies grown on solid media yielded inconsistent MIC data. This was attributed to the presence of uncontrolled numbers of coccus-shaped cells. In contrast, *Campylobacter* cells grown in broth cultures for 24 h were mainly spiral forms, and MIC data were more consistent. MICs for the *Campylobacter* reference strains used in this study were within the expected ranges (34). Consistent MICs were obtained after 24 h of incubation in duplicate trials. The MIC data shown in Table 2 represent the ranges for 30 *Campylobacter* strains. The results are based on data obtained after 24 h of incubation. After 48 h of incubation, MICs were 1 to 2 dilutions higher for some antibiotics, notably cephalothin, colistin, polymyxin, and rifampin. All test strains of *C. jejuni* were resistant to the antibiotics at the levels used in the selective media. The MICs of bacitracin, trimethoprim, and vancomycin for test and reference strains of *C. coli* were similar to those for *C. jejuni*. The resistance of *C. coli* to other antibiotics varied over a greater range than that of *C. jejuni* tested.

Based on the MIC data, a total of seven *C. coli* strains were selected for further study. The MICs of the antibiotics are shown in Table 3, except for bacitracin, trimethoprim, and vancomycin, which had MICs equivalent to those for *C. jejuni*. Three *C. jejuni* strains were also selected for comparison with the *C. coli* strains. Their antibiotic resistance was as follows: trimethoprim, 256 μg/ml; vancomycin, >128

TABLE 2. MICs of eight antibiotics for 24 *C. coli* strains and 6 *C. jejuni* strains

Antibiotics	MIC (μg/ml)[a] for:			
	C. coli			*C. jejuni* (range)
	Range	50%	90%	
Bacitracin[b]	>512	>512	>512	>512
Cephalothin	8–>256	256	>256	128–>256
Colistin[c]	2–32	8	16	4–32
Novobiocin	1–128	64	128	32–64
Polymyxin B sulfate[d]	0.5–8	2	8	2–8
Rifampin	2–>128	32	64	32–128
Trimethoprim	128–256	256	256	256
Vancomycin	>128	>128	>128	>128

[a] 50% and 90%, MICs for 50 and 90% of the strains, respectively.
[b] 1 mg/ml = 65.2 IU.
[c] 1 μg/ml = 13.6 IU.
[d] 1 μg/ml = 8 IU.

μg/ml; polymyxin B sulfate, 4 to 8 μg/ml; colistin, 8 to 16 μg/ml; rifampin, 32 to 128 μg/ml; cephalothin, 128 μg/ml; bacitracin, >512 μg/ml; and novobiocin, 16 to 32 μg/ml.

Effect of antibiotic combinations. The resistance of the selected *Campylobacter* strains to the combinations of antibiotics used in selective media was compared on MH agar. The number of colonies (CFU per milliliter) growing on MH agar was compared with the number of colonies growing on MH agar with antibiotic combinations. The differences in the $\log_{10}$ CFU per milliliter on MH agar and MH agar with antibiotics for each test culture with each antibiotic combination are shown in Table 4. The three *C. jejuni* strains grew well in the presence of the antibiotic combinations, except on media M5, M6, and M7. The greatest level of inhibition was a 5-log-cycle decrease in count for *C. jejuni* UA526 on medium M5. The addition of blood to media M5 and M6 reduced but did not eliminate the inhibitory effect.

The *C. coli* strains grew well on medium M1. In comparison to *C. jejuni*, however, they were inhibited by some of the other antibiotic combinations. Medium M7 caused the greatest inhibition. Counts for six of the seven strains were reduced by more than 7 log cycles. The cephalothin-susceptible strain (UA421) was inhibited by most antibiotic combinations. The addition of 10% sheep blood to media M5 and M6 reduced the inhibitory effect for some strains but failed to do so for others, e.g., the virtual elimination of the inhibitory effect of medium M5 on strains UA37 and UA44 as compared with no reduction in the inhibitory effect on strains UA40 and UA421.

As medium M7 was so inhibitory to the test strains, the effect of the addition of 10% sheep blood to the medium was studied. For those strains that exhibited intermediate to low levels of inhibition (1 to 4 log cycles), the addition of 10% sheep blood markedly reduced the inhibitory effect. For those strains that showed high levels of inhibition (7 log cycles), the addition of blood reduced the level of inhibition by 1 to 2 log cycles for strains UA37 and UA420, but there was less than a 1-log-cycle reduction of inhibition for other strains. Medium M7 was originally formulated with hemin (2 mg/liter) and 0.025% each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (31). Medium M7 was retested with these growth supplements instead of 10% sheep blood. Even though the counts were improved with the addition of hemin and ferrous sulfate-sodium metabisulfite-sodium pyruvate, five of the seven *C. coli* strains had counts that were still 3 to 5 log cycles lower than those on MH agar. The antibiotic combination used in medium M7 was more inhibitory to *C. coli* than to *C. jejuni*.

Strains that were inhibited by the antibiotic combinations (Table 4) were plated onto supplemented media for further evaluation with sheep blood or lysed horse blood in accordance with recommendations for the selective media (4, 5, 12, 15, 23, 25). The addition of blood to media M3, M4, M8, and M9 did not change the inhibitory effect of the antibiotic combinations in these media on strain UA421. However, for other strains of *C. coli*, the addition of blood to the media resulted in recovery improved by 2 to 5 log cycles. The addition of blood to medium M4 yielded variable results with strains UA40, UA44, and UA100. As many as seven replicates were done with these strains on medium M4 to study this variability. The mean $\log_{10}$ CFU per milliliter and the standard deviations for strains UA40, UA44, and UA100 are shown in Table 5. The standard deviations of counts on medium M4 with blood were higher than those on either MH agar or medium M4. Two batches of blood were not as effective as the others in improving the count on medium M4. Strain UA40 showed great variation in growth response on medium M4 with or without blood.

TABLE 4. Inhibitory effect of antibiotic combinations added to MH agar on *C. coli* and *C. jejuni*

Strain	Differences in $\log_{10}$ CFU/ml between the indicated media and MH agar[a]										
	M1	M2	M3	M4	M5	M5B[b]	M6	M6B[b]	M7	M8	M9
C. coli											
UA37	<1	<1	<1	<1	5	<1	<1	<1	>7	<1	<1
UA40	<1	<1	<1	7	>7	>7	>7	>7	>7	>7	<1
UA44	<1	4	<1	>7	>7	<1	1	<1	>7	3	<1
UA100	<1	<1	<1	5	5	<1	<1	<1	>7	2	<1
UA420	<1	<1	<1	2	1	<1	<1	<1	7	<1	<1
UA421	<1	<1	>7	6	>7	>7	>7	>7	>7	>7	>7
UA530	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
C. jejuni											
UA1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
UA124	<1	<1	<1	<1	<1	<1	2	<1	1	<1	<1
UA526	<1	<1	<1	<1	5	3	2	1	3	<1	<1

[a] Log count on MH agar minus log count on selective medium, corrected to the nearest log difference.
[b] M5B and M6B refer to media M5 and M6 with blood.

DISCUSSION

Primary isolation media should not be excessively inhibitory. However, for isolation as opposed to enumeration of bacteria, greater degrees of inhibition can be tolerated. Media used for the isolation of *Campylobacter* spp. depend on a range of antibiotics for their selective effect. This is necessary to control the competing microorganisms in stool specimens. In water and foods, in which *Campylobacter* spp. are likely to be present in lower numbers than other organisms, a similar if not more critical selection process is necessary.

The MICs of eight antibiotics used in the selective media were determined (Table 1). Amphotericin B and cyclohexi-

TABLE 3. Susceptibility of selected *C. coli* strains to polymyxin, colistin, rifampin, cephalothin, and novobiocin

Strain	MIC (μg/ml) of:				
	Polymyxin	Colistin	Rifampin	Cephalothin	Novobiocin
UA37	8	8–16	64–128	>256	128
UA40	2	8	2–4	64	2
UA44	0.5–2	4	16–32	128	32–64
UA100	8	8	16–32	128	64
UA420	2–4	16–32	32–64	>256	64
UA421	2–4	16	≤1–2	4–8	≤1
UA530	8	8–16	32–64	128–256	64

TABLE 5. Mean $\log_{10}$ CFU of *C. coli* UA40, UA44, and UA100 plated on MH agar, medium M4, and medium M4 with blood

Strain	Mean $\log_{10}$ CFU/ml ± SD on:		
	MH agar	Medium M4	Medium M4 with blood
UA40	8.26 ± 0.28	3.66 ± 2.98	5.14 ± 2.85
UA44	8.21 ± 0.12	2.05 ± 0.42	6.44 ± 1.86
UA100	9.14 ± 0.07	3.52 ± 1.01	7.33 ± 1.58

mide (Actidione) were excluded because they are added for their fungicidal effect and are not expected to have an effect on bacteria (3, 32). Cephalothin was substituted for other cephalosporins used in the original formulations (12, 31). The susceptibility of *Campylobacter* spp. to cephalosporins is similar (1, 14, 28); hence, this substitution should not affect the results. Saku et al. (24) observed no difference between MH agar with or without blood when the susceptibility of *Campylobacter* strains to 12 antibiotics was tested. However, serum proteins bind some antibiotics, such as novobiocin (2, 11, 14). This may account for the lower MICs of novobiocin observed on MH agar in this study. We observed that MH agar had no effect on trimethoprim activity, so it was not necessary to add lysed horse blood to media containing this antibiotic (6). The MICs of trimethoprim determined on blood-free MH agar in this study were similar to those in other reports (14, 20, 22).

MICs for *C. jejuni* are generally determined after 48 h of incubation (1, 28, 35). In fact, MICs should be read as soon as adequate growth is observed on control plates (30, 33). Prolonged incubation is not recommended for colistin, polymyxin, and rifampin (33). We used 24 h of incubation, and our results for the reference strains generally fell within the MIC ranges reported in the literature (34). Even though most strains of *C. coli* were resistant to the individual antibiotics used in the selective media, the antibiotic combinations often reduced the recovery of viable cells. The MICs of novobiocin and rifampin for the cephalothin-susceptible strain of *C. coli* used in this study (UA421) were also lower than those for the parent strain (UA420). About 2% of *C. coli* strains are reported to be susceptible to cephalothin (16).

The combination of antibiotics used in Skirrow (25) medium (M1), polymyxin B sulfate, trimethoprim, and vancomycin, had the least inhibitory effect on the *C. coli* and *C. jejuni* strains. In contrast, the most inhibitory medium (M7) contained cephalothin, polymyxin B sulfate, and rifampin. The concentration of polymyxin in medium M7 is eight times higher than that in medium M1. Lovett et al. (19) reported that high levels of polymyxin were inhibitory to many *C. jejuni* strains in their collection. Media that contained colistin in place of polymyxin, especially medium M6, in which a high concentration of bacitracin (25,000 IU/liter) was also used, inhibited the growth of *C. coli* strains. This might be expected, as polymyxin and colistin have similar modes of action (17). Other antibiotic combinations had an intermediate inhibitory effect on *C. coli* strains.

Added blood or growth supplements did not eliminate the inhibitory effect on *C. coli* strains. This supported our hypothesis that the growth of *C. coli* may be inhibited on media selective for *Campylobacter* spp. This could account for the lower incidence of isolation of *C. coli* than of *C. jejuni* from stool specimens obtained from patients with gastroenteritis. Although the addition of blood to some media reduced or eliminated the inhibitory effect of the antibiotic combinations, blood can cause variable and misleading results. This was seen with medium M4, in which an unaccountably poor recovery of cells was observed with two batches of blood. This effect convinced us of the desirability of developing or adapting a selective medium for *Campylobacter* spp. that does not rely on blood as an ingredient.

This study has indicated the possibility that some media used for the detection of *Campylobacter* spp. might inhibit the growth of *C. coli*. The antibiotic combinations used to suppress the growth of competing microorganisms in clinical specimens inhibit up to 10^7 *C. coli* cells grown in synthetic media. Although the strains used in this study might not represent isolates from clinical specimens, it is likely that they indicate a problem for enumerating or isolating *C. coli* from the extraenteric environment, such as water and foods.

ACKNOWLEDGMENTS

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

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