JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1983, p. 803–807
0095-1137/83/100803-05$02.00/0

Vol. 18, No. 4

Evaluation of Transport Media for *Campylobacter jejuni* in Human Fecal Specimens

WEN-LAN L. WANG,[1,2]* L. BARTH RELLER,[3] BETTY SMALLWOOD,[1] NANCY W. LUECHTEFELD,[1,2]† AND MARTIN J. BLASER[1,3]

Microbiology Laboratory, Denver Veterans Administration Medical Center, Denver, Colorado 80220,[1] and Department of Pathology[2] and Division of Infectious Diseases, Department of Medicine,[3] University of Colorado School of Medicine, Denver, Colorado 80262*

Received 2 May 1983/Accepted 13 July 1983

It is not always possible to culture feces immediately, and appropriate methods for transport of human specimens, unlike those from animals, have not been fully evaluated. Therefore, we took serial subcultures in two phases from six transport media inoculated with human diarrheal stools known to be positive for *Campylobacter jejuni*. In phase 1, Cary-Blair medium and buffered glycerol saline did not preserve *C. jejuni* as well as did alkaline peptone-water (APW), modified Cary-Blair medium, thioglycolate broth (Thio), and Campy-Thio. The four best media (APW, Cary-Blair medium, Thio, and Campy-Thio) preserved 20 fecal samples with *C. jejuni* better at 4°C (90% survival for 5 to 8 days) than at 25°C (90% survival for 1.7 to 2 days). In phase 2, APW and Thio, along with four modifications of the best media in phase 1, were tested with 23 positive strains. The ranges of survival times with modified media at 25°C were 1.3 to 2.2 days (90%) and 4.7 to 6.8 days (50%). APW with reducing agents preserved *C. jejuni* better than did APW alone, Thio plus ox bile, or Campy-Thio plus ox bile ($P < 0.05$). Thio at pH 8.5 was better at preserving *C. jejuni* than was APW or Thio plus ox bile ($P < 0.05$). If human fecal specimens cannot be refrigerated during transport or storage, we recommend the use of Thio at pH 8.5 or APW with reducing agents for preservation of *C. jejuni* at 25°C.

Campylobacter jejuni is a common cause of human enteritis and has been isolated from 3 to 14% of patients with diarrhea in Europe, North America, Australia, Africa, and Asia (3, 4). Antimicrobial agent-containing media or filtration methods that inhibit or eliminate normal flora have enabled the isolation of *C. jejuni* from fecal specimens (6, 14). Despite improved techniques, however, isolation of *C. jejuni* may still be difficult because of delays in culturing clinical specimens. Therefore, methods that maintain the viability of *C. jejuni* during transport and storage of human stool specimens are important, especially for specimens collected far from the processing laboratory.

We previously reported that modified Cary-Blair medium (MCB) was superior for transporting cecal specimens from healthy turkeys known to harbor *C. jejuni* (10). Since the normal cecal flora of turkeys differs from that in human feces and *Campylobacter* concentrations in animals with diarrhea may differ from those in healthy carriers, we were not certain whether MCB would also be adequate for specimens from humans with diarrhea. Therefore, we evaluated different media for their ability to preserve *C. jejuni* in fecal specimens from patients with diarrhea.

For our preliminary evaluation of transport media for human stool specimens, we studied alkaline peptone-water (APW) (11, 12), MCB (10), thioglycolate broth (Thio), Campy-Thio (C.Thio) (1), buffered glycerol saline (BGS) (8), and Cary-Blair medium (CB) (5). As with specimens from turkeys (10), we found that preservation was much more difficult for unrefrigerated human specimens. To improve survival, we modified APW and Thio and compared the results obtained when the original or modified media were used. In total, we compared 10 different transport media at room temperature (25°C). We found that two media, each of which was reduced and had an alkaline pH, enabled the best survival of *C. jejuni*.

MATERIALS AND METHODS

Source of specimens. All fecal specimens tested in this study were obtained from the clinical microbiology laboratories of Denver Veterans Administration Medical Center and University of Colorado Hospital. We used specimens from patients with diarrhea known

† Present address: 4001 W. Mesa Pass, Sioux Falls, SD 57106.

to be positive for *C. jejuni*. Eight positive stool specimens were used in the initial comparison of six media. Because of poor results obtained with BGS and CB, these media were not used in the analysis of 12 more specimens. Based on the results of this preliminary study of 20 specimens, we modified the media to be tested and used 23 more stool specimens for the second phase of the study.

Media. The transport media used for comparison and their sources are listed in Table 1. MCB was made by decreasing the agar in CB from 5.0 to 1.6 g/liter. C.Thio was made by adding antimicrobial agents to Thio as described previously (1). We modified APW by adding reducing agents, 0.05% sodium thioglycolate and 0.025% L-cystine (RAPW). The modified thioglycolate broth was made by changing the pH from 7.0 to 8.5 (Thio 8.5). We further modified Thio 8.5 and C.Thio by adding 1.5% ox bile to each (Thio-Bile and C.Thio-Bile).

Method of testing transport media. All stool specimens were screened for growth of *C. jejuni* by methods described previously (1). Positive stools were stored at 4°C for 24 to 72 h before being included in the study. A total of 1 g or 1 ml of the positive stool was mixed vortically with 9.0 ml of sterile distilled water. A 0.5-ml sample of this suspension was added to each of two 4.5-ml tubes containing each transport medium tested. One set of the inoculated transport media was placed in a refrigerator (4°C), and the other was left at room temperature (24 to 26°C). The survival of *C. jejuni* was monitored by subculturing 0.1 ml of the test medium onto Campy-BAP medium (1) every other day for 3 weeks and, thereafter, weekly until no growth was observed. In the last group of experiments with the reformulated media, we monitored growth daily. The plates were streaked with a standard bacteriological loop for the isolation of *C. jejuni* and incubated for 48 h at 42°C in a microaerobic atmosphere (5% oxygen, 10% carbon dioxide, 85% nitrogen). The endpoint of survival was defined as two consecutive negative cultures from the same tube of transport medium.

RESULTS

Table 2 shows the results of survival of *C. jejuni* in eight fecal specimens stored in six transport media at 4°C and room temperature.

TABLE 1. Transport media tested

Medium	Source
APW	Peptone, BBL Microbiology Systems, Cockeysville, Md. (pH 8.5)
CB	BBL
MCB	CB with decreased agar (1.6 g/liter)
BGS	BBL
Thio	PASCO, Denver, Colo.
C.Thio	PASCO
RAPW	APW plus 0.05% sodium thioglycolate and 0.025% L-cystine
Thio 8.5	Thioglycolate broth (pH 8.5)
Thio-Bile	Thioglycolate broth (pH 8.5) plus 1.5% ox bile
C.Thio-Bile	C-Thio (pH 8.5) plus 1.5% ox bile

TABLE 2. Survival of *C. jejuni* in eight human fecal specimens stored in six transport media at 4°C and room temperature

Medium	Survival (days) with refrigeration (4°C)			Survival (days) at room temp (24–26°C)		
	90%[a]	50%[a]	Range	90%[a]	50%[a]	Range
APW	4.2	33.0	4–52	1.8	7.0	1–7
CB	2.6	10.0	2–21	1.3	2.5	1–11
MCB	4.2	19.0	4–35	1.8	7.0	1–11
C.Thio	2.2	19.0	2–52	1.8	7.3	1–12
Thio	1.2	41.0	1–52	1.4	5.0	1–11
BGS	1.2	9.0	1–21	0.9	1.4	<1–2

[a] Percentage of specimens with viable *C. jejuni*.

At 4°C, half of the *C. jejuni* isolates were still alive at a low of 9 days in BGS and a high of 41 days in Thio. However, the 50% survival time of *C. jejuni* in these media at room temperature (24 to 26°C) ranged from only 1.4 days (BGS) to 7.3 days (C.Thio). Because BGS and CB consistently gave the worst results, these media were eliminated from subsequent trials.

Figure 1 compares the four best transport media that we continued to test with a total of 20 stool specimens. The 90% survival time of *C. jejuni* in these specimens kept at 4°C ranged from 5 to 8 days, and 50% of the isolates were still viable in all four media after 2 weeks. The ranges in days for each medium were as follows: APW, 4 to 93; MCB, 4 to 36; C.Thio, 2 to 53; and Thio, 1 to 69. At room temperature (Fig. 2), however, the 90% survival times were short (1.7 to 2.0 days), with ranges in days as follows: APW, 1 to 10; MCB, 1 to 12; and C.Thio and Thio, 1 to 13.

Figure 3 shows the survival of *C. jejuni* in 23 human specimens in APW, Thio, and four reformulated media at room temperature. The 90% survival times ranged from 1.3 to 2.2 days, and 50% of the isolates were alive at 4.7 to 6.8 days. The ranges in days were as follows: APW, 1 to 12; RAPW, 1 to 15; Thio, 1 to 15; Thio 8.5, <1 to 18; Thio-Bile, <1 to 14; and C.Thio-Bile, <1 to 12.

Comparisons of media with the Wilcoxon matched-pair, signed-ranks test showed that RAPW preserved *C. jejuni* better than did APW, Thio-Bile, or C.Thio-Bile, and Thio 8.5 was better than APW or C.Thio-Bile. All of these differences were significant at the 0.05 level. Thio 8.5 and RAPW appeared to preserve *C. jejuni* better than did Thio, but the differences in survival time were not statistically significant.

DISCUSSION

The need for special incubation conditions to isolate *C. jejuni* from stool specimens has made it difficult for some small hospitals to culture

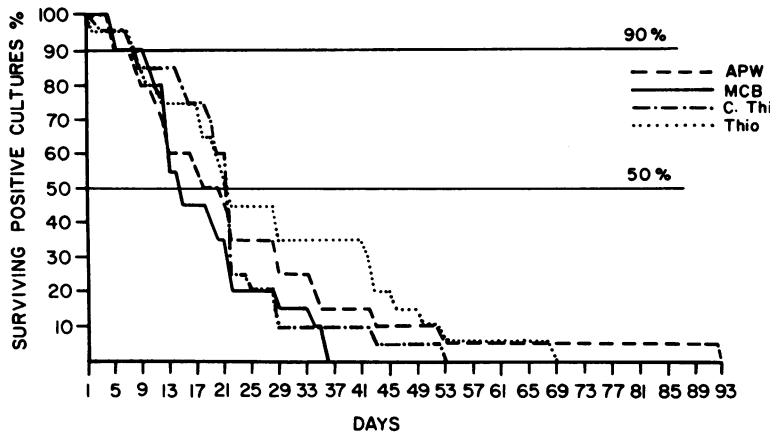

FIG. 1. Survival of *C. jejuni* in 20 human fecal specimens stored in four transport media at 4°C. Percentages refer to the number of specimens with viable *C. jejuni*.

these organisms. Furthermore, during outbreak investigations and field studies, stool specimens from patients with diarrhea frequently are transported to a distant laboratory for isolation of *C. jejuni*. Therefore, the development of a satisfactory transport medium is important. Our previous studies showed that cecal specimens from healthy turkeys survived best in MCB. However, the microbial flora in ceca of healthy turkeys with a body temperature of 42°C was quite different from the fecal contents of humans with diarrhea (W.-L. L. Wang, unpublished data). Thus, this study was undertaken to determine the survival of *C. jejuni* in human fecal specimens in different transport media at 4°C and at room temperature.

BGS, recommended for preservation of fecal specimens for culturing species of *Salmonella* and *Shigella* (8), sustains the viability of *Salmonella* and *Shigella* species. The absence of nutrients in BGS prevents the overgrowth of the usual enteric flora. *Salmonella* and *Shigella* species then may be isolated with the use of selective media. In our previous (12) and present studies, however, *C. jejuni* perished rapidly in BGS. Saline is known to inhibit growth of *C. jejuni* (7), a mechanism that may explain this phenomenon.

The survival of *C. jejuni* at 4°C in various transport media was relatively long: 90% survival for 5 to 8 days and 50% survival for at least 2 weeks. The present results agreed with our previous studies (2) that showed a longer survival of *C. jejuni* in stools kept at 4°C (9 to 28 days).

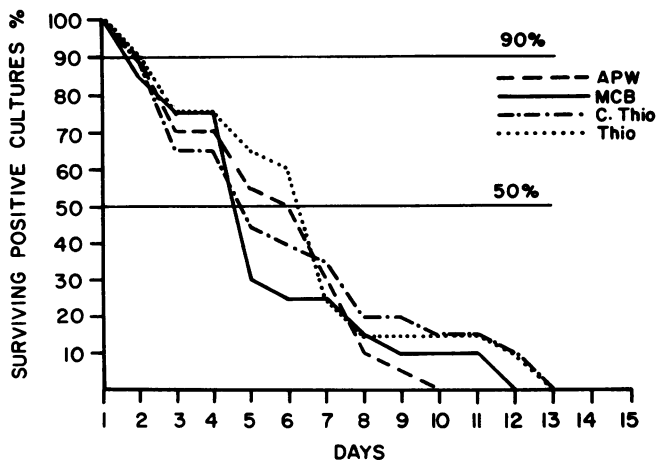

FIG. 2. Survival of *C. jejuni* in 20 human fecal specimens stored in four transport media at room temperature (24 to 26°C). Percentages refer to the number of specimens with viable *C. jejuni*.

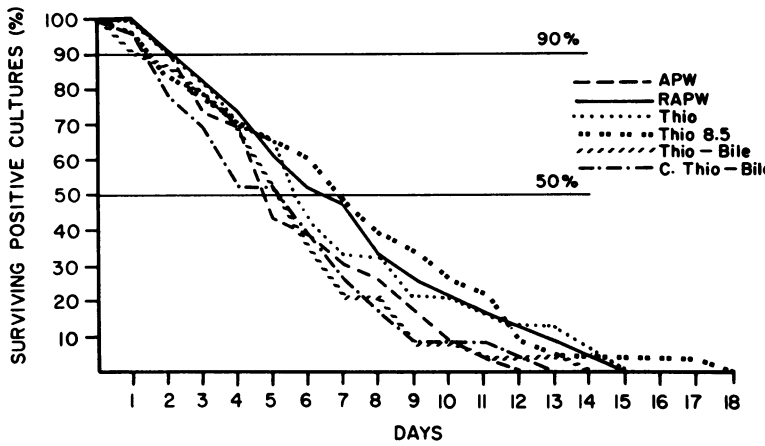

FIG. 3. Survival of *C. jejuni* in 23 human fecal specimens stored in six modified transport media at room temperature (24 to 26°C). Percentages refer to the number of specimens with viable *C. jejuni*.

Our early quantitative study showed a gradual loss of *C. jejuni* in each specimen, even within 1 to 3 days; the present results which showed some loss of *C. jejuni* within a few days at 4°C are consistent with these findings. The more rapid loss of *C. jejuni* in our previous study may have been due to our not using effective transport media.

Our studies at 4°C showed that APW sustained *C. jejuni* as well as did MCB, Thio, or C.Thio. CB (0.5% agar) did not maintain the organism well, which was similar to the results reported by Lior and Krol (Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C174, p. 339). The survival of *C. jejuni* in MCB (0.16% agar) in our study was not as good as that obtained by Lior and Krol, who used fluid CB. The presence of small amounts of agar in our MCB, as well as the differences in technique, may account for the differences in the survival times of *C. jejuni*. We used naturally infected stool specimens, whereas the other investigators used fecal specimens that had been inoculated with *C. jejuni* strains maintained in the laboratory. If only one medium can be used for transport of fecal specimens, MCB with decreased agar could be used since CB has been commonly used for transporting specimens containing species of *Salmonella* and *Shigella* (5).

The survival of *C. jejuni* at room temperature (Fig. 3) was less than desired. RAPW and Thio 8.5 appeared to sustain the organisms better than the other four media, and their use prolonged median survival time by 1 to 2 days. These two media share the characteristics of being alkaline and reduced. An increase in survival time of *C. jejuni* by RAPW or Thio 8.5 from 4 or 5 to 6 or 7 days could be clinically significant.

Osteroom et al. found that the addition of ox bile (1.5%) improved the chance of isolation of *C. jejuni* from human feces, animal feces, and other sources (13). Earlier, we demonstrated that *C. jejuni* survived well in bile and multiplied when maintained at 37°C (2). However, the addition of bile to Thio did not result in improved survival of *C. jejuni* at room temperature.

George et al. (9) demonstrated that *C. jejuni* grew better in brucella broth when reducing agents were present. Our addition of thioglycolate and cystine to APW also prolonged the survival of *C. jejuni*. It would be interesting to determine whether the use of ferrous sulfate, sodium metabisulfite, and sodium pyruvate, instead of thioglycolate and cystine, would further prolong the survival of *C. jejuni* in APW.

We diluted naturally infected stool specimens that had been in the refrigerator for 1 to 3 days. Thus, the survival of *C. jejuni* in undiluted fresh human stools in transport medium may actually be longer than the survival times found in our experiments. If stool needs to be preserved for future use, our data show that storage at 4°C, which gave a median survival of at least 2 weeks in all media tested except CB and BGS, would provide the best chance of survival for *C. jejuni*. For shipment of stool specimens at room temperature, Thio 8.5 or RAPW would be better choices.

ACKNOWLEDGMENTS

This work was supported in part by the Veterans Administration Medical Research Service.

We thank Evelyn Fitzgerald for typing this manuscript.

LITERATURE CITED

1. **Blaser, M. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, B. Reller, and W.-L. L. Wang.** 1979. Campylobacter en-

teritis: clinical and epidemiologic features. Ann. Intern. Med. **91**:179–185.
2. **Blaser, M. J., H. L. Hardesty, B. Powers, and W.-L. L. Wang.** 1980. Survival of *Campylobacter fetus* subsp. *jejuni* in biological milieus. J. Clin. Microbiol. **11**:309–313.
3. **Blaser, M. J., and L. B. Reller.** 1981. Campylobacter enteritis. N. Engl. J. Med. **305**:1444–1452.
4. **Butzler, J. P., and M. B. Skirrow.** 1979. Campylobacter enteritis. Clin. Gastroenterol. **8**:737–765.
5. **Cary, S. G., and E. B. Blair.** 1964. New transport medium for shipment of clinical specimens. I. Fecal specimens. J. Bacteriol. **88**:96–98.
6. **Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon.** 1972. Acute enteritis due to related vibrio: first positive cultures. J. Infect. Dis. **125**:390–392.
7. **Doyle, M. P., and D. J. Roman.** 1982. Response of *Campylobacter jejuni* to sodium chloride. Appl. Environ. Microbiol. **43**:561–565.
8. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed., p. 7–8. Burgess Publishing Co., Minneapolis, Minn.
9. **George, H. A., P. S. Hoffman, R. M. Smibert, and N. R. Krieg.** 1978. Improved media for growth and aerotolerance of *Campylobacter fetus*. J. Clin. Microbiol. **8**:36–41.
10. **Luechtefeld, N. W., W.-L. L. Wang, M. J. Blaser, and L. B. Reller.** 1981. Evaluation of transport and storage techniques for isolation of *Campylobacter fetus* subsp. *jejuni* from turkey cecal specimens. J. Clin. Microbiol. **13**:438–443.
11. **Monsur, K. A.** 1963. Bacteriological diagnosis of cholera under field conditions. Bull. W.H.O. **28**:387–389.
12. **Mosley, W. H.** 1970. Principles and practice of cholera control, p. 26. World Health Organization, Geneva.
13. **Osteroom, J., M. J. G. M. Vereijken, and G. B. Engels.** 1981. Campylobacter isolation. Vet. Q. **3**:104.
14. **Skirrow, M. B.** 1977. Campylobacter enteritis. A "new" disease. Br. Med. J. **2**:9–11.