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# Evaluation of Filters for Recovery of *Campylobacter jejuni* from Water

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*Campylobacter jejuni* has been incriminated in several large waterborne outbreaks, but it has rarely been isolated from water itself. Better methodology is needed for the isolation of *C. jejuni* from water. We evaluated three types of 0.45-μm microporous filters and three different pore sizes of positively charged depth filters for their ability to recover *C. jejuni* from seeded, sterile tap and surface water. The microporous filters tested were Millipore HA, Gelman GN6, and Zetapor. Three pore sizes of Zeta Plus depth filters (05S, 30S, and 50S) were evaluated by using an adsorption-elution technique. The overall percent recovery in both tap and surface water by microporous filters was: Zetapor, 66%; Millipore HA, 33%; and Gelman GN6, 33%. Adsorption-elution with Zeta Plus 50S allowed 89% recovery of *C. jejuni*. These data suggest that both the positively charged Zetapor microporous filter and the Zeta Plus 50S depth filter are effective filters for the recovery of *C. jejuni* from water.

*Campylobacter jejuni* is now well established as an important etiological agent of gastroenteritis (1, 5, 6). In several studies, it was isolated more often than *Salmonella* spp. or *Shigella* spp. from patients with diarrhea (1, 4, 5). The epidemiology of *C. jejuni* diarrhea is beginning to be elucidated. It has become apparent that there is a vast animal reservoir of this organism and that transmission occurs by the fecal-to-oral route. Food and water are the vehicles most often incriminated during outbreaks (3).

In this country, at least two large waterborne outbreaks have been reported, affecting ca. 4,300 people (3, 7). In these outbreaks, *C. jejuni* was implicated by its isolation from individuals with diarrhea, but the organism could not be isolated from the incriminated water sources. One of the probable reasons for this inability to isolate the organism from water is that the isolation methodology for *C. jejuni* was designed to detect the organism in the feces of ill individuals who shed $10^6$ to $10^9$ cells per g. These methods are not sensitive enough to detect small numbers of *C. jejuni* in large volumes of water.

*C. jejuni* probably occurs commonly in surface waters since many domestic animals and waterfowl have been shown to shed this agent in their feces. Also, any water contaminated with human wastes has the potential to contain *C. jejuni*. This is especially important, since this organism has been reported to remain viable in water for up to 5 weeks (2), and although little is known about the minimum infective dose of *C. jejuni* for humans, it is believed to be low. To study the occurrence of *C. jejuni* in water, better methods of concentration from large volumes of water are needed. The purpose of this study was to compare the ability of three different types of microporous filters (0.45 μm) to recover *C. jejuni* from seeded, sterile surface and tap water. We also sought to evaluate the ability of three different pore sizes of positively charged depth filters to recover *C. jejuni* from surface and tap water by using an adsorption-elution technique.

## MATERIALS AND METHODS

**Evaluation of microporous filters.** Four strains of *C. jejuni* isolated from patients with acute gastroenteritis were used in this study. The three 0.45-μm microporous filters evaluated were Millipore HA (MP; Millipore Corp., Bedford, Mass.), Gelman GN6 (GL; Gelman Instrument Co., Ann Arbor, Mich.), and Zetapor (ZP; AMF/Cuno, Meriden, Conn.). Each strain of *C. jejuni* was grown for 24 h on Campy blood agar at 42°C in an atmosphere of 5% oxygen. This growth was diluted in sterile 0.85% saline to a concentration of 50 organisms per ml. Spread plates on Campy blood (Scott/Randolph Laboratories, Houston, Tex.) agar were done in triplicate to determine the number of *C. jejuni* in the suspension that was to be added to the sterile water samples. One milliliter of each of the *C. jejuni* suspensions was added to each of six 99-ml aliquots of sterile tap water dechlorinated with 0.5 ml of 1 M sodium thiosulfate and to each of six 99-ml aliquots of sterile surface water. A 100-ml sample of seeded water was passed through each of the three types of filters. This was done in duplicate with both surface and tap water. Filters were placed on Campy blood agar and incubated at 42°C for 48 h in 5%

oxygen. The colonies of *C. jejuni* were counted. All filters were tested twice with each strain in both tap and surface water, and the average was used to calculate the percent recovery.

**Evaluation of Zeta Plus depth filters.** Three different pore sizes of Zeta Plus filters (AMF/Cuno) were studied: 05S, 30S, and 50S (larger numbers indicate smaller average pore size). All depth filters were used in a 47-mm Tri-47 filter holder (AMF/Cuno). Overnight growth of two strains of *C. jejuni* was diluted to >250 organisms per ml in 0.85% saline. One milliliter of this *C. jejuni* suspension was added to each of six 99-ml aliquots of sterile, dechlorinated tap water and each of six 99-ml aliquots of sterile surface water. A 100-ml sample was passed through each of the different Zeta Plus filters. The filter holder was inverted and the adsorbed *C. jejuni* cells were eluted by passing 10 ml of 7.2 pH Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) through the inverted filter. The number of *C. jejuni* in the influent, effluent, and eluent was determined by the spread plate technique. Each trial was done in duplicate for each strain, and the averages of two trials per strain were used to calculate the percent recovery. The differences in percent recovery of *C. jejuni* among filters and strains were compared by using analysis of variance.

## RESULTS

Table 1 shows the percent recovery of the four strains of *C. jejuni* by microporous filters in both sterile tap and surface water. ZP consistently had a higher recovery than MP or GL, and this difference was significant ($P < 0.005$). In addition, strains that had a tendency to swarm on MP and GL formed more distinct colonies on ZP. The inoculum used in this set of experiments showed that 25 to 50 *C. jejuni* cells per 100 ml could be detected by all of the microporous filters tested. The differences in recoveries for the four strains tested were also significant ($P < 0.0005$). The percent recovery of two strains of *C. jejuni* by the different pore sizes of Zeta Plus depth filters in both sterile tap and surface water is given in Table 2. Only the smallest pore size tested (50S) allowed no *C. jejuni* to pass through the filter. Also, the 50S had a total recovery of 88.5%. The two larger pore sizes (05S and 30S) had a much lower recovery of *C. jejuni*. This adsorption-elution technique required an inoculum of >250 *C. jejuni* cells per 100 ml to detect *C. jejuni* because of the use of 10 ml of eluent and the use of only 0.1 ml of culture on the spread plate. This excess dilution would not be a problem in a system that employed an enrichment broth.

TABLE 1. Recovery of *C. jejuni* from water by microporous filters

| Strain | % Recovery by: | | | | | |
|---|---|---|---|---|---|---|
| | GL[a] | | MP[b] | | ZP[c] | |
| | Tap | Surface | Tap | Surface | Tap | Surface |
| 1 | 28[d] | 5 | 31 | 14 | 52 | 48 |
| 2 | ND[e] | 21 | 10 | 26 | 50 | 39 |
| 3 | 0 | 54 | 7 | 73 | 37 | 99 |
| 4 | 70 | 48 | 42 | 59 | 95 | 78 |

[a] Means: tap, 33; surface, 33; overall, 33.
[b] Means: tap, 22; surface, 43; overall, 33.
[c] Means: tap, 59; surface, 66; overall, 63.
[d] Average of two trials, each done in duplicate.
[e] Average could not be determined because of swarming.

TABLE 2. Percent recovery of two strains of *C. jejuni* by three pore sizes of Zeta Plus depth filters

| Pore size | Percent | | | | | |
|---|---|---|---|---|---|---|
| | Recovery | | Passing through | | Adsorbed | |
| | Tap | Surface | Tap | Surface | Tap | Surface |
| 05S | 35.0[a] | 24.2 | 27.1 | 8.3 | 37.9 | 67.5 |
| 30S | 20.0 | 16.0 | 10.7 | 4.0 | 69.3 | 80.0 |
| 50S | 88.9 | 88.1 | 0.0 | 0.0 | 11.1 | 11.9 |

[a] Average of two trials per strain, each done in duplicate.

## DISCUSSION

In this study, we found that ZP allowed significantly better recovery of *C. jejuni* than did the other microporous filters tested (GL and MP). These three microporous filters have the same pore size (0.45 μm). The major difference between ZP and the other two microporous filters is that ZP has a net positive surface charge. It is probable that *C. jejuni*, like most gram-negative bacteria, has a slight net negative surface charge. Microporous filters with a negative charge retain bacteria by physical entrapment. ZP can retain bacteria by both physical and charge interactions, which probably accounts for their more efficient recovery of *C. jejuni* along with the inhibition of swarming by this organism. Strain-dependent differences in recoveries suggest that surface charge variations exist in *C. jejuni*. Furthermore, as new methods are developed, they must be evaluated against a battery of *C. jejuni* strains.

The results from the evaluation of the microporous filters were the reason that positively charged depth filters were evaluated in the second part of this study. Depth filters, especially in cartridge form, can increase the volume of water sampled, thus increasing the likelihood of detecting low numbers of organisms. This study was performed to determine which filters are best able to recover *C. jejuni*.

It was found that positively charged ZP and Zeta Plus depth filters (50S) had the highest percent recovery of *C. jejuni* from spiked samples of both sterile tap and surface water. The use of microporous filters or depth filters for the

isolation of *C. jejuni* would depend on several factors, such as the volume of the sample to be tested, the turbidity of the water, the suspected level of *C. jejuni* contamination, and whether an enrichment culture technique was to be used. We used sterile water samples in this study to study the recovery efficiency of these filters. In the actual isolation of *C. jejuni* from most waters, a selective enrichment culture procedure may be necessary to reduce the number of competing microorganisms. To determine the optimal methodology for the isolation of this organism from water, enrichment media will have to be evaluated.

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