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Evaluation of Coliphage Detection as a Rapid Indicator of Water Quality

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A rapid coliphage analysis technique for enumerating coliphages in natural waters has been evaluated by water quality laboratories located throughout the United States. Correlations were established between coliphages and coliforms in natural water systems. These correlations were highly significant. This relationship can thus be used to determine the number of fecal or total coliforms present in natural water samples based on an enumeration of coliphages. With this method, coliphages in natural water systems (containing ≥six coliphages per 100 ml) can be enumerated within 6 h.

The importance of coliphages as indicators of fecal contamination in water was first recognized by Guelin (2, 3). In this early work, Guelin observed a correlation between the coliform and coliphage levels (2, 3). Since that time, many researchers have developed methods for enumerating coliphages in water. Several attempts have also been made to establish a relationship between coliforms and their corresponding coliphages. The literature on bacteriophages as indicators of bacterial and viral contamination of water has been reviewed by Scarpino (5). In the review, Scarpino stated that "correlations appear to exist in fresh and marine waters between fecal bacterial pathogens, such as *Salmonella* and *Shigella* species, and fecal indicator bacteria, such as *Escherichia coli*, and their bacteriophages."

In 1969, a rapid test was developed by Atlantic Research Corp. for determining the presence of *Shigella, Salmonella*, and *E. coli* in water. The test determined the number of bacteriophages of a given bacterial species in water samples within a 4- to 6-h period. The procedure was based on the addition of a dense culture of bacteria to a portion of the water sample, followed by plating of the sample with a nutrient agar medium. Bacteriophages were enumerated by counting the clear areas (plaques) in the bacterial lawn. Of particular concern was the presence of *E. coli*. Quantitative relationships between fecal or total coliform bacteria and coliphages were established during this work (4).

Subsequent research has been aimed at developing this rapid test for use in water quality laboratories. Since only coliforms and their corresponding coliphages are of interest for these applications, the test was given a new name, ARCAT (a rapid coliphage analysis technique). As the ARCAT test developed, it was recognized that the coliform-coliphage correlation established earlier should be verified over a wider geographical area and time span. The need for user comments on the test methodology was also recognized. To gain this information, we undertook a test and evaluation program.

This paper presents the results of the evaluation of the ARCAT procedure by 13 water quality laboratories located in cities throughout the United States. The cities involved in the study analyzed a variety of waters. Of the 13 cities, 10 collected natural water samples from streams, rivers, lakes, and reservoirs. Two of the cities sampled water through their municipal water treatment plants. Three other cities sampled chlorine-treated wastewater. Since only limited chlorine-treated wastewater data were collected, a reliable statistical analysis of these data could not be made. Therefore, the chlorine-treated wastewater data were not included in this paper.

MATERIALS AND METHODS

Samples. Water samples were collected and analyzed by the 13 participating city water quality laboratories over the time period from May to November 1978. Approximately 1,000 water samples were collected for analysis. The analyses included an enumeration of the fecal and total coliform levels by either the standard membrane filter or the most-probable-number technique, an enumeration of coliphage levels by the ARCAT test, and a determination of pH, dissolved oxygen, turbidity, and residual chlorine (if applicable) by standard methods. The cities participating in this evaluation, the types of water that they sampled, and the mean pH, turbidity, and dissolved oxygen values are listed in Table 1.

Media and culture preparation. Media for the ARCAT procedure were prepared as follows. The 20× nutrient broth was prepared by dissolving 160 g of nutrient broth (Difco Laboratories) and 100 g of NaCl

TABLE 1. Water quality laboratories participating in the evaluation of the ARCAT test, the type of water sampled, and average values for pH, turbidity, and dissolved oxygen[a]

Location of water quality laboratory	Type of water sampled	Avg pH	Avg turbidity (NTU)	Avg dissolved oxygen (ppm)
Akron, Ohio	Natural	7.7	1.0	6.3
Arlington, Tex.	Natural	7.7	1.3	8.4
Chicago, Ill.	Natural	8.2	2.8	6.8
Clearwater, Fla.	Chlorine-treated wastewater	7.3	1.5	ND
Dallas, Tex.	Natural	7.5	ND	ND
Ft. Lauderdale, Fla.	Natural	7.3	2.9	5.9
Janesville, Wis.	Chlorine-treated wastewater	7.3	ND	7.7
Little Rock, Ark.	Water treatment	7.2	1.4	ND
Minneapolis, Minn.	Water treatment	8.3	4.5	ND
Nashville, Tenn.	Natural and chlorine-treated wastewater	7.5	ND	6.7
Philadelphia, Pa.	Natural	7.2	6	ND
Worcester, Mass.	Natural	7.5	1.2	ND
Fairfax County, Va.	Natural	7.0	8.2	7.1

[a] NTU, Nephelometric turbidity units; ND, not determined.

(ACS grade, Fisher Scientific Co.) in glass-distilled water to a volume of 1 liter. The resultant broth was sterilized by autoclaving for 15 min at 15 lb/in^2.

Soft nutrient agar was prepared by dissolving 8.0 g of nutrient broth (Difco), 10.0 g of agar (Difco), and 5.0 g of NaCl (ACS grade, Fisher) in glass-distilled water to 1 liter and sterilizing the resultant solution.

Nutrient agar plates were prepared by dispensing 20 ml of hot sterile nutrient agar into plastic petri dishes (15 by 100 mm). The agar was made by dissolving 23 g of nutrient agar (Difco) and 5 g of NaCl (ACS grade, Fisher) in glass-distilled water. After dilution to 1 liter, the mixture was sterilized, cooled, and poured.

The bacterial strain used in the ARCAT test for this evaluation study was *E. coli* C (ATCC 13706). The host culture of this organism was prepared by inoculating from a slant into 500 ml of sterile nutrient broth. This broth was prepared by dissolving 8 g of nutrient broth (Difco) in glass-distilled water to make 1 liter, followed by sterilization. The culture was allowed to incubate unshaken at 37°C for 18 h.

ARCAT procedure. The ARCAT procedure used to enumerate the coliphages in the water samples is shown in Fig. 1. A 100-ml amount of the water sample was placed in a sterile polyethylene cup. Five milliliters of sterile 20× nutrient broth (160 g of nutrient broth plus 100 g of NaCl per liter) and 10 ml of the host culture were mixed with the water sample (total volume, 115 ml). Four 5-ml aliquots were removed from the cup. Each aliquot was added to a test tube containing 3 ml of sterile, melted soft nutrient agar (8 g of nutrient broth, 10 g of agar, and 5 g of NaCl per liter) maintained at 45°C. Four drops of the 18-h host culture were added to each tube to enhance the formation of the bacterial lawn. The contents of each tube were then mixed and poured onto a petri dish containing a sterile solid agar layer. The top agar layer was allowed to harden; the plates were then inverted and placed in a 37°C incubator for 4 to 6 h. The numbers of plaques on the plates were counted at 4 and 6 h. The number of coliphages in the original 100-ml water sample was obtained by summing the plaques on the four plates and multiplying by 5.75 (to account for broth and host culture added to the sample). For waters containing high levels of contamination, dilutions of the water sample were prepared with sterile distilled water.

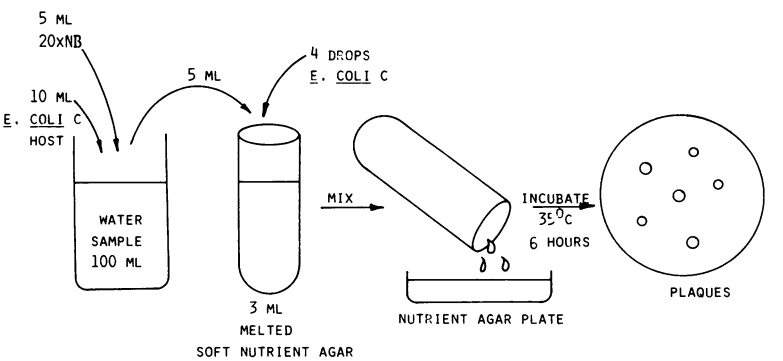

FIG. 1. ARCAT test protocol. NB, Nutrient broth.

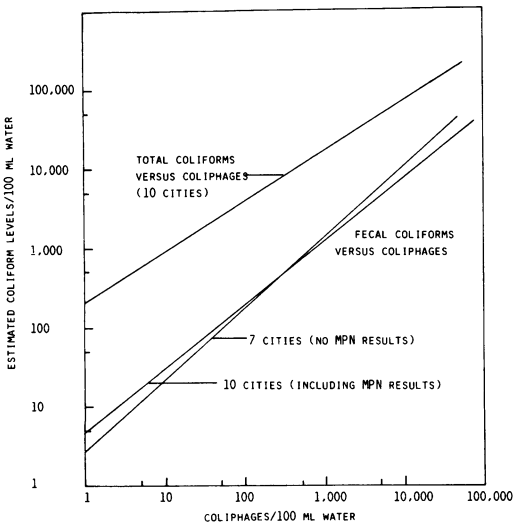

FIG. 2. Coliform-coliphage relationship from 1978 data. MPN, most probable number.

The coliphage, fecal, and total coliform data from each laboratory were sent to Atlantic Research Corp. for statistical analysis. For this analysis, the data were separated into natural and treated water samples. The statistical analysis consisted of transforming the data to $\log_{10}$ for homogeneity of variance. A straight line was then fit through the data by the least-squares method. Lines were calculated for fecal coliforms versus coliphages and total coliforms versus coliphages. Analysis of variance tables were calculated to determine whether the lines were significant at the 99.9% level.

RESULTS AND DISCUSSION

Natural waters analyzed. Approximately 600 natural water samples were collected and analyzed for fecal coliforms, total coliforms, and coliphages. The data from these assays were plotted as log-log plots of fecal coliforms or total coliforms versus coliphages. The least-squares lines, which were fit to the data for the 6-h plaque counts, are shown in Fig. 2. The statistical analysis of these samples is presented in Table 2. Correlation coefficients for the 6-h counts were 0.69 for fecal coliforms versus coliphages and 0.62 for total coliforms versus coliphages. These correlations were significant at the 99.9% level, indicating a high probability of a relationship between fecal or total coliforms and coliphages in natural waters. For the 4-h readings, the sensitivity (the number of coliphages detected) was decreased, although the slopes of the lines were close to those obtained with the 6-h data. Thus, 4-h ARCAT data can be used to determine fecal and total coliform levels in water, when sensitivity can be sacrificed for the sake of time.

During the analysis, problems were uncovered in data from three of the cities. Two of the cities used the most-probable-number method for determining fecal and total coliforms. The results of the most-probable-number procedures were presented as "less than a number" for the fecal coliform levels. This type of data was not precise enough for a reliable comparison with the ARCAT results. The third city experienced problems in the detection of fecal coliforms at their major sampling site. With the elimination of the data from these three cities, the correlation between fecal coliforms and coliphages was significantly improved (Table 2 and Fig. 2).

The natural water data for fecal coliforms and coliphages from this program are plotted in Fig. 3, along with natural water data from an earlier series of tests. The earlier data were obtained by analyzing 50 water samples in the Washington, D.C., area during the period from January to March 1974 and were reported in the literature (4). The slopes of the natural water lines are approximately the same; however, the intercepts are different, indicating a difference in test sensitivity. The sensitivity difference is due to the difference in sample size plated and counted for coliphages. In the earlier tests, 50 ml of each sample was plated and counted (4), thus allow-

TABLE 2. Statistical analysis of natural water samples

No. of cities	Plate incubation (h)	Fecal coliform level vs coliphage level[a]							Total coliform level vs coliphage level[a]						
		No. of samples	Correlation coefficient	F value	Log_{10} b_0	b_0 intercept	Log_{10} b_1	b_1 slope	No. of samples	Correlation coefficient	F value	Log_{10} b_0	b_0 intercept	Log_{10} b_1	b_1 slope
All 10	4	600	0.68	524[b]	0.97	9.333	0.79	6.166	621	0.62	397[b]	2.54	346.7	0.59	3.890
All 10	6	533	0.69	539[b]	0.69	4.898	0.80	6.310	611	0.62	388[b]	2.30	199.5	0.61	4.074
7	4	454	0.76	611[b]	0.72	5.248	0.87	7.413							
7	6	443	0.76	600[b]	0.45	2.818	0.89	7.762							

[a] b_0, Intercept of regression line; b_1, slope of regression line. Predicted coliforms = $b_0 + b_1 \times$ coliphages per 100 ml.

[b] Significant at the 99.9% level.

TABLE 3. Coliforms and coliphages through Little Rock's water treatment system

Settled water		Filtered water		Potable water		
Total coliforms	Coliphages	Total coliforms	Coliphages (1 h, 6 h)[a]	Total coliforms	Coliphages 1 h, 6 h[a]	Coliphages 2 h, 6 h[b]
0	23	0	12	0	0	
0	23	0	0	0	0	
0	0	0	1,035	0	0	0
0	0	0	0	0	0	0
0	138	0	6	0	0	23
0	69	0	6	0	0	23
0	57	0	23	0	0	0
9	69	0	207			
0	23	0	35			
0	35	0	46			
0	35	0	0			
0	104					
0	138					
0	57					
0	360					
0	0					
0	0					

[a] A 1-h incubation of the water sample, nutrients, and the host, followed by plating of 20 ml of the mixture and incubation of the plates for 6 h.

[b] A 2-h incubation of the water sample, nutrients, and the host, followed by plating of 20 ml of the mixture and incubation of the plates for 6 h.

ing the detection of two coliphages per 100 ml. However, in this study, only 17.4 ml of the water sample was plated, yielding a detection limit of 5.7 coliphages per 100 ml. Thus, the earlier procedure was approximately 2.87 times more sensitive. The similarity in the slopes of the lines indicates that the correlation between fecal coliforms and their corresponding coliphages in natural waters is constant over time, over wide geographical areas, and over a variety of natural waters. Thus, fecal and total coliform levels in natural water systems can be estimated by an enumeration of coliphages and an application of the correlation.

TABLE 4. Minneapolis water treatment

Water	Total coliforms per 100 ml	Coliphages per 100 ml
Softened	22	0
	17	23
	4	29
	130	80
	49	69
	49	86
	2	12
	23	0
	170	17
	79	17
	110	29
	240	29
	250	23
Filtered	1.8	6
	1.8	23
	1.8	0
Potable	1.8	1[a]
	1.8	0
	1.8	0
	1.8	23

[a] A 1-h incubation of the water sample, nutrients, and the host, followed by plating of 20 ml of the mixture and incubation of the plates for 6 h.

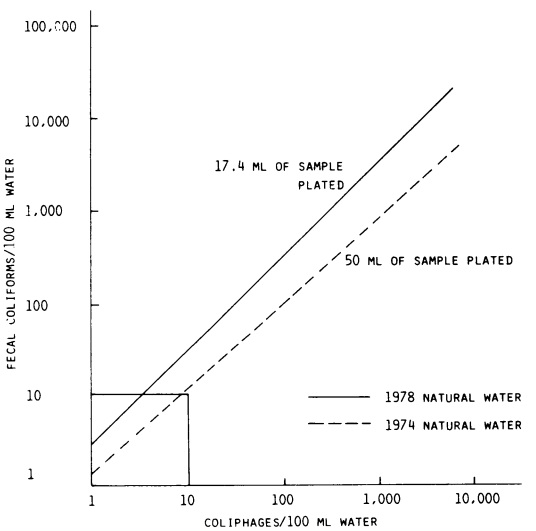

FIG. 3. Comparison of the relationships of fecal coliforms to coliphages from two field test programs with natural water samples.

Treated water samples. An additional aspect of the study was to test water samples through water treatment systems to determine whether coliphages were present. Two of the cities sampled and analyzed water through their municipal water treatment systems: Little Rock, Ark., and Minneapolis, Minn. To improve the sensitivity of the ARCAT test to potable water levels, the test samples were incubated with the host cells and nutrients for 1 or 2 h before plating. This procedure allowed any coliphage in the sample to invade a bacterium, reproduce, and release large numbers of coliphages into the sample. Thus, the chance of detection of a single coliphage in the 100-ml sample was greatly improved. Data obtained from these cities are shown in Tables 3 and 4. These data show that coliphages can be found routinely in filtered water in which no total coliforms are found. Coliphages were also found in some potable water samples. These data confirm the results of other investigators. For example, Shuval (6) stated that conventional water treatment technology effectively reduces or removes coliforms, but is not capable of reliably removing viruses under all conditions. Berg (1) indicated that coliforms appear to be destroyed more rapidly than viruses by disinfection.

Conclusions. Natural water samples from the 10 cities were analyzed for fecal and total coliforms and coliphages detected by the broad-range *E. coli* C host. The data were analyzed statistically to determine whether a relationship existed between fecal or total coliforms and coliphages. An analysis of the 6-h ARCAT data and the fecal and total coliform data indicated that a linear relationship exists between coliphages detected with the *E. coli* C host and fecal or total coliforms. The correlation coefficients of the least-squares line fit to the data were 0.69 for fecal coliforms versus coliphages and 0.62 for total coliforms versus coliphages. These correlations are significant at the 99.9% level.

The results of the study indicated that the ARCAT procedure is a rapid, inexpensive method for enumerating coliphages in waters containing $\geq$ six coliphages per 100 ml. The coliphage levels can then be related to coliforms to give a quick indication of the number of coliform bacteria in potentially contaminated waters. The ARCAT method as described in this paper is applicable to coliphage analysis natural water systems containing $\geq$ six coliphages per 100 ml. However, for the monitoring of potable water systems, the sensitivity of the test must be improved so that one coliphage per 100 ml can be detected.

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