

IDEXX

Literature Cover Sheet

IDEXX #: 8A

Title: Absence of Association between Total Heterotrophic and Total Coliform Bacteria from a Public Water Supply

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Source: Applied and Environmental Microbiology

Topic: Heterotrophic Plate Count(HPC) correlation with Total Coliforms(TC)

Highlights:

- Analysis of water samples over a 2 year period showed the distribution water system HPC was not statistically associated with TC.

- The appearance of HPC or TC did not predict the appearance of the other within 10 days of isolation.

- There was no association between season and HPC or TC.

- It appears to be premature to use HPC data to predict the occurrence of TC.

* See page 380 & page 383.

Absence of Association between Total Heterotrophic and Total Coliform Bacteria from a Public Water Supply

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* Heterotrophic plate counts (HPC) and total coliforms (TC) are two major microbial indicators that are used to monitor the potability of water. Although the presence of heterotrophs has been hypothesized to predict the presence of TC, there have been few documented reports. Intensive sampling of raw, treated effluent and distribution water from a public water supply serving 400,000 people provided an opportunity to study the relationship between these two indicator groups of bacteria. A total of 26,158 samples were analyzed, including 12,970 from 1986 and 13,188 from 1985. There were 13,429 samples from the distribution system, 5,524 from treatment effluents, and 7,205 from raw water. The associations between HPC and TC were made on both a hits-and-misses and numerical comparison (CFU per milliliter) basis. The periodicity of the two indicators was also analyzed to determine whether the presence of one group could predict the presence of the other. Atypical bacteria were also related to the presence of these two indicator bacteria. Venn diagrams and nonparametric statistics revealed the following correlation coefficients for HPC and TC for 1985 and 1986 combined: raw water $r = 0.45$, treated effluent $r = 0.06$, and distribution system $r = 0.10$. Atypical bacteria showed a similar relationship with HPC. There was no predictive periodicity for HPC and TC within ± 10 days of isolation of each other. Therefore, in a 2-year survey of a public water supply, the presence of HPC had a low correlation coefficient with TC, as determined by hits-and-misses and numerical comparisons. The enumeration of one group was found to be independent of the other.

There are several possible indicators of microbial contamination of water. The first are the infectious agents themselves. Unfortunately for the analyst, they are not present in potable water in sufficient numbers for their direct enumeration to be possible. Therefore, laboratories have relied on the enumeration of sentinals or indicators to predict the presence of the primary agents. Of the primary sentinals most useful in predicting mammalian colonic contamination of water, *Escherichia coli* has been the most extensively used. Because it was difficult early in this century to directly detect *E. coli* (or other intracolonic microorganisms) and differentiate it from other enteric bacteria, a class of secondary sentinals was developed. These are known as total coliforms (TC) and include lactose-fermenting, gas-producing, or *o*-nitrophenyl- β -D-galactopyranoside (ONPG) positive, oxidase-negative members of the family *Enterobacteriaceae* (1, 12).

An additional indicator of water quality is the heterotrophic plate count (HPC), previously known as the standard plate count. This measure is an attempt to provide a single value that expresses the number of aerobic and facultatively anaerobic microorganisms in a water sample (1, 20). The heterotrophic group of bacteria encompass an extremely broad range of genera, and these bacteria are defined by the medium utilized in their enumeration (16, 23).

Although many investigators have examined the presence of either heterotrophs or TC isolated from drinking water (7, 13, 17) and water treatment plants (5, 6, 8, 15), few investigators have attempted to relate them. LeChevallier et al. (14) found that there was an association between heterotrophs and *Aeromonas* species in a potable water system but did not demonstrate an association between *Aeromonas* species and TC. Reilly and Kippin showed that HPC had no relationship

with coliform numbers in the Beverly-Salem system below 50 HPC/ml (21), and Geldreich et al., using a different system, showed a relationship at high HPC levels (10). LeChevallier et al. found a correlation between HPC and various physical, chemical, and bacteriological determinations (16). Goshko et al., in studying distribution water in the Philadelphia area, found that the association between HPC and TC varied with the individual water system (11).

There is a common belief that an increase in HPC antedates an increase in TC (10, 11, 14, 21). Accordingly, many utilities believe that the addition of increased disinfectant when the heterotrophic populations begin to increase will prevent TC occurrences. The purpose of this study was to examine the relationship between heterotrophs and TC and determine whether the enumeration of one could predict the presence and elimination of the other.

MATERIALS AND METHODS

Bacteriological procedures. All procedures were in compliance with American Public Health Association guidelines (1). All TC analyses were performed by the membrane filtration technique with Millipore filters and equipment (Millipore Corp., Bedford, Mass.). Atypical microorganisms were considered nonsheer (red) colonies grown on m-Endo agar plates. Heterotrophic bacteria were determined on R2A agar by the spread plate technique (1). There are a number of methods for performing HPC or standard plate count tests (1). While individual methods may yield different numerical results, the results of this study in relation to other HPC methods should not change, except possibly in degree.

Data base. Our data base consisted of all TC and HPC analyses performed in 1985 and 1986. A total of 26,158 samples were examined, of which 7,205 were from raw water, 5,524 were from treated effluents, and 13,188 were from the distribution system. In addition, routine physical

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and chemical analyses were performed on each of these samples.

Analyses. To determine whether a relationship existed between the presence of TC and heterotrophs, we analyzed the data in several ways. First, the presence of one target group was compared with the presence of the other. Second, to account for the possibility that large numbers of one target group might influence the density (CFU per milliliter) of the other, the results of all bacterial analyses for each water sample were divided into numerical category data. Third, periodicity was evaluated to determine whether the presence of either target group could antedate the presence of the other. The lead-lag duration chosen to evaluate periodicity was 10 days forward and 10 days backward. Fourth, associations were examined to determine whether there was a seasonal component to the relationship between the two groups of target microorganisms. Lastly, because atypical (red, nonsheened colonies on m-Endo agar) colonies were commonly recovered, the data were analyzed to determine whether they were correlated with either TC or heterotrophs.

Statistical analyses. Statistical analyses were performed by commonly accepted procedures (4, 9), taking into account the skewed distribution of both parametric and nonparametric data found in examining water samples (11).

RESULTS

Hits-and-misses analysis. To examine the relationship between TC and heterotrophs, we initially evaluated the data base for its distribution. It was apparent that it was highly skewed, with a great majority of samples yielding no growth. Because the data were skewed, it appeared appropriate to dichotomize them for hits-and-misses analysis. Correlations between TC and heterotrophs were made by using a number of nonparametric statistics based on chi-squared analysis. In addition, the occurrence of atypical microorganisms was also included in a second series of analyses.

The results of the chi-square analyses are best presented visually in the form of Venn diagrams. In the Venn diagram, the area of the square represents the total number of samples that occurred in the data set undergoing analysis. The area of each circle represents the number of positive hits, or occurrences, for a particular target microorganism group. Figure 1 presents the analysis for raw, treated effluent and distribution water in 1986. Results were substantially the same in 1985. In 1986, the raw-water correlation between TC and heterotrophs was 0.44, a moderate correlation. The P value ($P = <0.01$), indicates that these results were unlikely to occur by chance alone. For the treatment system the overlap is much less, as is the general number of positive specimens for both the TC and HPC categories. The correlation coefficient, $r = 0.063$, between these two groups was poor. For the distribution system, the correlation coefficient between TC and HPC was 0.076. In 1985 the correlation coefficients for the two target groups were 0.049 in raw water, 0.066 in the treatment system samples, and 0.115 in the distribution system samples.

The results of the hits-and-misses analysis for the entire period of study showed that except in raw water, for which there was moderate correlation ($r = 0.45$) between HPC and TC, there were very weak correlation coefficients, below 0.12, in distribution and treatment system samples. Other measures of the relationship between these two indicator groups including sensitivity, specificity, false-positive rate, and false-negative rate, corroborate the correlation coeffi-

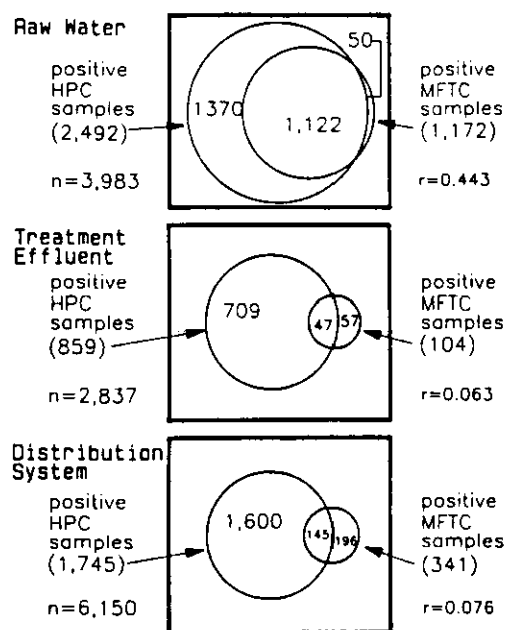


FIG. 1. Correlation of TC and HPC from all water sources studied in 1986. The total area of the Venn diagram represents the total number of samples. The overlap represents the number of hits, and the extralapl represents the number of misses.

icients. Accordingly, the analysis of the 1985 and 1986 data bases indicated that the presence of either HPC or TC in the treated effluents was unlikely to predict the presence of these groups in the distribution system (Fig. 1).

The data were also examined to determine whether there was a seasonal correlation between HPC and TC during the year. It was considered possible that water temperature and other factors would provide the proper environmental conditions for an association to occur that might be masked by analysis of the full-year data. In 1985 the proportion of positive counts for both HPC and TC varied by season. The largest proportion for TC was observed in the summer, when 14% of the samples were positive compared with an average of 3 to 4% for the rest of the year. For HPC the proportion ranged from 18% in the spring to 26% in the summer, with the second highest proportion being observed in the winter, at 23%. Therefore, TC appeared to be more strongly associated with water temperature than HPC were. In 1986 the proportions were of similar magnitudes but showed more consistent trends, with the highest proportion for both TC and HPC observed in the summer, followed by spring and winter (Table 1).

Numerical category data. To determine whether the numbers of CFU per milliliter for either TC or HPC affected the numbers or isolation rate of the other, each numerical occurrence was placed into a numerical category. Comparison cells for the data were constructed for 1985 and 1986. These comparison cells permitted the data to be examined on a direct numerical category basis. Table 2 presents the numerical category data for the distribution system in 1986. The results from the numerical category data closely paralleled those observed in the hits-and-misses analysis. Except for raw water, there was a weak correlation between the isolation of HPC and TC. The actual number of CFU counted in either group had no effect on the occurrence of the other. Finally, it was observed that the number of TC or

TABLE 1. Correlation of TC and HPC by season for the distribution system and treatment effluents

Source	Yr and season	No. of samples	% Positive HPC	% Positive TC	Correlation of HPC and TC
Treatment effluent	1985		26.6	4.8	0.069
	Winter	406	34.5	1.5	0.126
	Spring	782	31.1	2.2	0.014
	Summer	783	23.1	12.4	0.125
	Fall	700	20.9	1.0	0.090
	1986		30.3	3.7	0.063
	Winter	681	30.4	2.6	0.030
	Spring	730	31.4	3.3	0.041
	Summer	729	25.4	5.5	0.136
	Fall	697	34.1	3.2	0.043
Distribution system	1985		20.8	4.9	0.074
	Winter	1,195	22.7	3.3	0.148
	Spring	2,181	18.0	3.8	0.088
	Summer	1,781	23.6	10.3	0.055
	Fall	1,912	20.1	2.0	0.020
	1986		28.4	5.5	0.076
	Winter	1,900	20.4	2.3	0.072
	Spring	1,631	29.5	5.9	0.101
	Summer	1,312	32.6	9.2	0.076
	Fall	1,307	34.4	6.2	0.008

HPC did not predict or have any influence on the density of bacteria from the other group.

Periodicity. To determine whether the presence of either HPC or TC could predict the periodicity of the other, the data were analyzed by relating lead-lag time with correlation coefficients. For the occurrence of each group at a point in time, the occurrence of the other group was examined for a period of 10 days before and 10 days after isolation occurred. Correlation coefficients based on this analysis were generated for 1985 and 1986. Figure 2 presents the correlation coefficients for 1985 and 1986. Again, there was a weak correlation coefficient between the isolation of HPC and TC. Within this weak correlation coefficient, the highest values were found within 2 days of isolation of each other ($r = 0.03$ to 0.22). In no case was a negative correlation coefficient greater than marginal found. Therefore, the results from the periodicity analysis indicate that the occurrence of HPC does not predict the occurrence of TC and that the occurrence of TC does not predict the occurrence of HPC, within ± 10 days.

Relationship of atypical microorganisms. As previously noted, atypical bacteria were frequently isolated on m-Endo agar throughout the study. Because this group is largely defined as one of exclusion (either nonshreen or nonconfirming colonies from m-Endo agar), there is some question of whether the atypical microorganisms should be thought of as a coliform variant or some other entity. The association between HPC and atypical microorganisms was made by considering the total m-Endo agar colony count. Their relationship to HPC was determined on a hits-and-misses basis by the nonparametric techniques described above. The correlation of atypical microorganisms with HPC was similar to that observed with TC alone compared with HPC bacteria (Fig. 3). The correlation coefficients were remarkably similar to those seen in Fig. 1. Total m-Endo agar plate counts demonstrated a moderate correlation of 0.385 in the 1985 to 1986 period in raw water and less than 0.13 for 1985 and 1986

TABLE 2. Numerical category analysis for the distribution system in 1986^a

TC (row) vs HPC (column)	Frequency	Row %	Column %	Expected value	Deviation	Cell chi square
0	4,209	4,160.8	48.2	0.559406	72.46	95.55
1-5	800	840.7	-40.7	1.96584	13.77	89.89
6-10	172	178.5	-6.5	0.238162	2.96	91.01
11-50	233	231.4	1.6	0.01085	4.01	95.10
51-100	59	61.4	-2.4	0.0935	1.02	90.77
101-200	57	59.5	-2.5	0.105605	0.98	90.48
>201	279	276.8	2.2	0.018228	4.80	95.22
0	165	192.7	-27.7	3.97485	61.34	3.75
1-5	70	38.9	31.1	24.8004	26.02	7.87
6-10	8	8.3	-0.3	0.008612	2.97	4.23
11-50	8	10.7	-2.7	0.688493	2.97	3.27
51-100	4	2.8	1.2	0.47077	1.49	6.15
101-200	4	2.8	1.2	0.561947	1.49	6.35
>201	10	12.8	-2.8	0.618658	3.72	3.41
0	10	18.6	-8.6	3.99254	38.46	0.23
1-5	9	3.8	5.2	7.29026	34.62	1.01
6-10	4	0.8	3.2	12.8234	15.38	2.12
11-50	2	1.0	1.0	0.897625	7.69	0.82
51-100	0	0.3	-0.3	0.247497	0.00	0.00
101-200	1	0.3	0.7	2.02092	3.58	1.59
>201	0	1.2	-1.2	1.2387	0.00	0.00
0	20	29.4	-9.4	2.98755	48.78	0.45
1-5	8	5.9	2.1	0.71985	19.51	0.90
6-10	4	1.3	2.7	5.95841	9.76	2.12
11-50	2	1.6	0.4	0.082313	4.88	0.82
51-100	2	0.4	1.6	5.6641	4.88	3.08
101-200	1	0.4	0.6	0.800952	2.44	1.59
>201	4	2.0	2.0	2.14446	9.76	1.37
0	0	2.1	-2.1	2.14878	0.00	0.00
1-5	2	0.4	2.6	15.1645	100.00	0.34
6-10	3	0.1	-0.1	0.092195	0.00	0.00
11-50	0	0.1	-0.1	0.119512	0.00	0.00
51-100	2	0.0	-0.0	0.031707	0.00	0.00
101-200	1	0.0	-0.0	0.030732	0.00	0.00
>201	0	0.1	20.1	0.142927	0.00	0.00
0	1	1.4	-0.4	0.130591	50.00	0.02
1-5	1	0.3	-0.3	0.289431	0.00	0.00
6-10	2	0.1	-0.9	14.3313	50.00	0.53
11-50	1	0.1	-0.1	0.079675	0.00	0.00
51-100	0	0.0	20.0	0.021138	0.00	0.00
101-200	1	0.0	-0.0	0.020488	0.00	0.00
>201	0	0.1	-0.1	0.095285	0.00	0.00

^a Chi square, 113.709 (df = 30, $P = 0.000$); Phi, 0.136; Contingency coefficient, 0.135; Cramer V, 0.061; Likelihood ratio, 80.494 (df = 30, $P = 0.000$); Mantel-Haenszel chi-square, 0.059 (df = 1, $P = 0.809$).

in the treated effluents and distribution system samples. Therefore, atypical microorganisms were shown to closely parallel TC and be correlated with HPC to virtually the same extent.

DISCUSSION

TC and HPC have been used for many years to assess the microbiological and sanitary quality of water. They have been shown to exist within the sediments and surfaces of water distribution pipes. It appears that coliforms, owing to unknown factors, can assume a lifestyle like that of the heterotrophs and persist until conditions favorable to their existence are removed (2, 3, 6, 18, 22, 24).

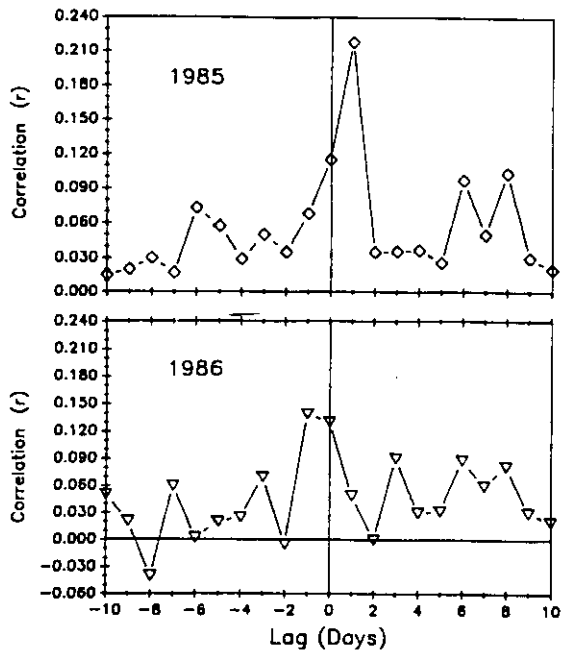


FIG. 2. Periodicity between the isolation of TC and HPC.

Epidemiological studies of the relationship between HPC and TC have shown mixed results. In one of the first studies to examine the relationship between these two indicator groups, Geldreich et al. (10) demonstrated that there appeared to be an increased occurrence of TC above an HPC count of 31/ml. However, between 31 and greater than 1,000 HPC per ml the TC occurrences, expressed as percent positive samples, did not increase.

In studying the microbiological quality of water in the distribution systems in the neighboring towns of Salem and

Beverly, Mass., which are served by a common water source but have independent distribution systems, Riley and Kippin found that each system demonstrated its own ecological characteristics (21). They attributed this primarily to the newer pipes in the Beverly system, which seemed to be more refractory to the establishment of endogeneous bacterial populations than the pipes in the older Salem system. They showed that HPC counts from 0 to 50 CFU/ml had no correlation with TC isolations. When the counts exceeded 50 CFU/ml, TC numbers increased, although there were few analyses in this category. They concluded that low HPC counts did not affect the frequency of total coliform recovery and that coliforms may become part of the endogenous flora of the distribution system and act like HPC, and that "high or low HPC densities do not indicate either the presence or absence of coliforms" (21).

Goshko et al. (11) studied the correlation of TC occurrence with HPC and other physical and chemical parameters in a large number of water systems in the Philadelphia, Pa., area. They found that each water system demonstrated its own relationship between HPC and TC. Of the seven systems studied, three demonstrated a positive correlation coefficient between HPC and TC, whereas one demonstrated a negative correlation coefficient between these two target groups. They concluded that their results were similar to those of Geldreich et al. (10) in that above 30 HPC/ml there was no statistically correlated association between HPC and TC. Later, in studying four local Philadelphia water systems, they found that HPC increased with hydraulic residence in the distribution system and that this factor might account for the observed increase in coliform occurrences with increasing HPC (H. A. Minnigh, W. O. Pipes, G. I. Ramirez-Toro, and W. D. Rosenzweig, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, N37, p. 250).

The analyses of intensive water sampling undertaken during a 2-year period have shown that in a distribution water system HPC was not statistically associated with TC. It was found that there was a poor correlation between the isolation of HPC and TC either on a hits-and-misses basis or on the basis of numerical categories. Furthermore, it was found that the occurrence of either HPC or TC did not antedate or predict the appearance of the other within 10 days of isolation. There was also no association between season and HPC or TC. Lastly, atypical microorganisms were more closely associated with HPC than with TC. From this analysis and those in the literature, it appears premature to use HPC data to predict the occurrence or activity of TC. Each water system may be unique in its ecology and may have to be analyzed individually to determine the TC-HPC relationship.

ACKNOWLEDGMENTS

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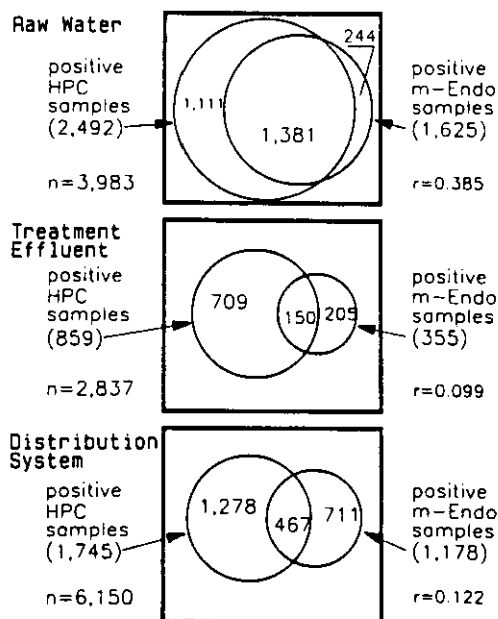


FIG. 3. Correlation of atypical microorganisms and HPC from all water sources. The Venn diagrams are explained in the legend to Fig. 1.

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The results of the chi-square analyses are best presented visually in the form of Venn diagrams. In the Venn diagram, the area of the square represents the total number of samples that occurred in the data set undergoing analysis. The area of each circle represents the number of positive hits, or occurrences, for a particular target microorganism group. Figure 1 presents the analysis for raw, treated effluent and distribution water in 1986. Results were substantially the same in 1985. In 1986, the raw-water correlation between TC and heterotrophs was 0.44, a moderate correlation. The P value ($P = <0.01$), indicates that these results were unlikely to occur by chance alone. For the treatment system the overlap is much less, as is the general number of positive specimens for both the TC and HPC categories. The correlation coefficient, $r = 0.063$, between these two groups was poor. For the distribution system, the correlation coefficient between TC and HPC was 0.076. In 1985 the correlation coefficients for the two target groups were 0.049 in raw water, 0.066 in the treatment system samples, and 0.115 in the distribution system samples.

The results of the hits-and-misses analysis for the entire period of study showed that except in raw water, for which there was moderate correlation ($r = 0.45$) between HPC and TC, there were very weak correlation coefficients, below 0.12, in distribution and treatment system samples. Other measures of the relationship between these two indicator groups including sensitivity, specificity, false-positive rate, and false-negative rate, corroborate the correlation coeffi-

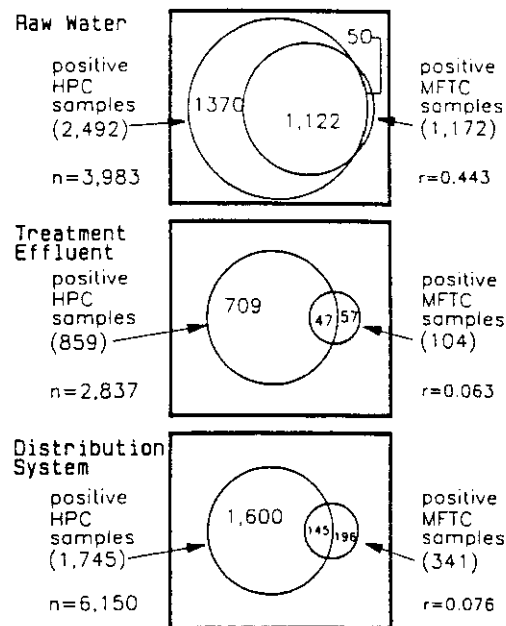


FIG. 1. Correlation of TC and HPC from all water sources studied in 1986. The total area of the Venn diagram represents the total number of samples. The overlap represents the number of hits, and the extralap represents the number of misses.

cient. Accordingly, the analysis of the 1985 and 1986 data bases indicated that the presence of either HPC or TC in the treated effluents was unlikely to predict the presence of these groups in the distribution system (Fig. 1).

The data were also examined to determine whether there was a seasonal correlation between HPC and TC during the year. It was considered possible that water temperature and other factors would provide the proper environmental conditions for an association to occur that might be masked by analysis of the full-year data. In 1985 the proportion of positive counts for both HPC and TC varied by season. The largest proportion for TC was observed in the summer, when 14% of the samples were positive compared with an average of 3 to 4% for the rest of the year. For HPC the proportion ranged from 18% in the spring to 26% in the summer, with the second highest proportion being observed in the winter, at 23%. Therefore, TC appeared to be more strongly associated with water temperature than HPC were. In 1986 the proportions were of similar magnitudes but showed more consistent trends, with the highest proportion for both TC and HPC observed in the summer, followed by spring and winter (Table 1).

Numerical category data. To determine whether the numbers of CFU per milliliter for either TC or HPC affected the numbers or isolation rate of the other, each numerical occurrence was placed into a numerical category. Comparison cells for the data were constructed for 1985 and 1986. These comparison cells permitted the data to be examined on a direct numerical category basis. Table 2 presents the numerical category data for the distribution system in 1986. The results from the numerical category data closely paralleled those observed in the hits-and-misses analysis. Except for raw water, there was a weak correlation between the isolation of HPC and TC. The actual number of CFU counted in either group had no effect on the occurrence of the other. Finally, it was observed that the number of TC or

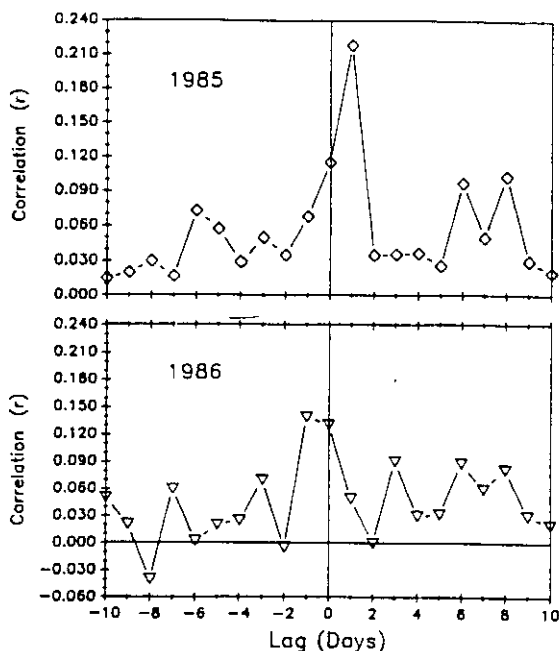


FIG. 2. Periodicity between the isolation of TC and HPC.

Epidemiological studies of the relationship between HPC and TC have shown mixed results. In one of the first studies to examine the relationship between these two indicator groups, Geldreich et al. (10) demonstrated that there appeared to be an increased occurrence of TC above an HPC count of 31/ml. However, between 31 and greater than 1,000 HPC per ml the TC occurrences, expressed as percent positive samples, did not increase.

In studying the microbiological quality of water in the distribution systems in the neighboring towns of Salem and

Beverly, Mass., which are served by a common water source but have independent distribution systems, Riley and Kippin found that each system demonstrated its own ecological characteristics (21). They attributed this primarily to the newer pipes in the Beverly system, which seemed to be more refractory to the establishment of endogenous bacterial populations than the pipes in the older Salem system. They showed that HPC counts from 0 to 50 CFU/ml had no correlation with TC isolations. When the counts exceeded 50 CFU/ml, TC numbers increased, although there were few analyses in this category. They concluded that low HPC counts did not affect the frequency of total coliform recovery and that coliforms may become part of the endogenous flora of the distribution system and act like HPC, and that "high or low HPC densities do not indicate either the presence or absence of coliforms" (21).

Goshko et al. (11) studied the correlation of TC occurrence with HPC and other physical and chemical parameters in a large number of water systems in the Philadelphia, Pa., area. They found that each water system demonstrated its own relationship between HPC and TC. Of the seven systems studied, three demonstrated a positive correlation coefficient between HPC and TC, whereas one demonstrated a negative correlation coefficient between these two target groups. They concluded that their results were similar to those of Geldreich et al. (10) in that above 30 HPC/ml there was no statistically correlated association between HPC and TC. Later, in studying four local Philadelphia water systems, they found that HPC increased with hydraulic residence in the distribution system and that this factor might account for the observed increase in coliform occurrences with increasing HPC (H. A. Minnigh, W. O. Pipes, G. I. Ramirez-Toro, and W. D. Rosenzweig, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, N37, p. 250).

The analyses of intensive water sampling undertaken during a 2-year period have shown that in a distribution water system HPC was not statistically associated with TC. It was found that there was a poor correlation between the isolation of HPC and TC either on a hits-and-misses basis or on the basis of numerical categories. Furthermore, it was found that the occurrence of either HPC or TC did not antedate or predict the appearance of the other within 10 days of isolation. There was also no association between season and HPC or TC. Lastly, atypical microorganisms were more closely associated with HPC than with TC. From this analysis and those in the literature, it appears premature to use HPC data to predict the occurrence or activity of TC. Each water system may be unique in its ecology and may have to be analyzed individually to determine the TC-HPC relationship.

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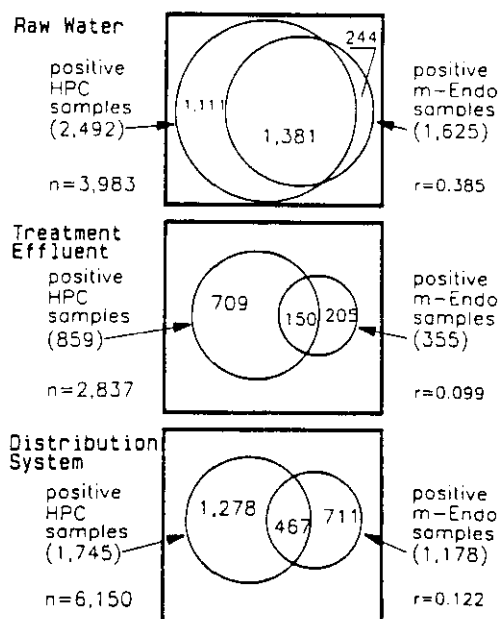


FIG. 3. Correlation of atypical microorganisms and HPC from all water sources. The Venn diagrams are explained in the legend to Fig. 1.