# Coliphage Association with Coliform Indicators: A Case Study—Brazil

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## Abstract

Many microbiological tests are currently available for evaluating the suitability of water resources for human use. Cost, speed, simplicity, and the ability of the test to detect microbial contamination are some of the key factors involved in selecting the appropriate test. The performance of the test often depends on the nature of the tested water and hence it is necessary to evaluate the test under local conditions. This paper compares the performance of several microbiological tests on Brazilian waters. These tests include traditional coliform tests, and the presence/absence and coliphage tests.

## INTRODUCTION

In order to reduce the risk of consuming contaminated water, it is essential to monitor the microbiological quality of potable water according to a well-defined strategy. The objectives of microbiological water quality should be used to define the water sources to be monitored, the frequency of sampling, the bacteriological tests to be used, and the reporting of the results. This is especially important in developing countries where the financial and technical resources are lacking. Perhaps the most important factor in devising this monitoring strategy is to choose the bacteriological tests that can quickly and economically assess the microbiological quality of potable and raw waters.

This project is part of a major program on the development of a classification system for potable water sources in Southeast Asia, Africa, and South America, which is being funded by the International Development Research Centre (IDRC), Ottawa. The IDRC provides

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direct financial support to the local research team, and also provides microbiological and statistical consultant support to assist the researchers to primarily evaluate the coliphage test as an indicator of sanitary quality of potable water sources and secondly to evaluate several simple, inexpensive bacteriological techniques to assess drinking water quality.

This paper summarizes the results of the IDRC project in Brazil, and also presents a comparison and evaluation of a number of water quality assessment methods for use with drinking water. These studies encompass both drinking and raw waters. The range of water types included cover the spectrum of the Brazilian potable water sources and hence the results will provide a realistic assessment of the applicability of these techniques on the national level.

## METHODS

### Water Samples

A total of 112 drinking water samples were collected from different sources, and assessed using the presence/absence (P/A), $H_2S$ paper strip, MF-Endo total coliform, and coliphage tests. The samples are distributed as follows: (1) 100 samples from the City of São Paulo and the surrounding area, with 81 of these samples collected from chlorinated water and the remaining 19 from well waters; (2) 12 samples from bottled drinking water.

A total of 162 raw water samples prior to treatment were collected in triplicate from 6 major drinking water plants from October 1986 to June 1987. The samples were tested using fecal coliform MPN, membrane filter, and coliphage techniques.

### Coliphage Tests

The procedure described by Wetsel *et al.* (1982) and reproduced in Section 919C of the American Public Health Association's (APHA) *Standard Methods* (1985) with the addition of 2,3,5-triphenyl tetrazolium chloride and using *Escherichia coli* C (ATCC no. 13706) as host was used in this study [American Society for Testing and Materials ASTM,1982].

### Microbiological Tests

Raw water samples were subjected to the following APHA *Standard Methods* (1985) total coliform and fecal coliform tests: the five-tube MPN procedure using lauryl tryptose broth and brilliant green lactose bile broth with fecal coliform confirmation in EC broth; the five-tube MPN procedure using A-1 broth and the membrane filtration fecal coliform procedure using modified fecal coliform (M-FC) agar for stressed bacteria with 0.45-$\mu$ membrane filter.

All drinking water samples were tested by the P/A test (Clark, 1969), and all positive tests were subjected to confirmation tests for total coliforms, fecal coliforms, fecal streptococci, *Clostridium spp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Aeromonas* spp., as detailed by Clark *et al.* (1982). The drinking water samples were also tested by the $H_2S$ paper strip technique using chemically inoculated paper strips as described by Manja *et al.* (1982). All positive samples by the $H_2S$ procedure were subjected to similar identification procedures (for Enterobacteriaceae and *Clostridium*) as used in the P/A test. Total coliforms counts using M-Endo agar and membrane filtration (APHA, 1985) were also carried out on all potable water samples.

### Chemical Tests

Free residual chlorine was assessed in all chlorinated potable water samples using the APHA *Standard Methods* (1985).

### Statistical Methods

Nonparametric statistical methods were used to evaluate the association and or the equivalence of the bacteriological methods. These include Spearman's rank correlation and the McNemar test. The importance of temporal variabilities were assessed using the one-way analysis of variance technique.

## RESULTS AND DISCUSSION

### Raw Water

Table I presents a typical set of data that was used in the statistical analyses. Table II summarizes all the coliphage, and total and fecal coliform, data obtained from the raw water samples. This table reveals that the standard deviation exceeds the mean (the coefficient of variation exceeds 2.5) and the median is smaller than the mean. These findings indicate that the microbiological characteristics of the raw water sources vary substantially and that the distribution pattern is highly skewed to the left.

The association between each pair of microbiological characteristics was measured using Spearman's rank correlation test. The results

TABLE I
Microbiological results—Raw water sources that supply water treatment plants of the greater São Paulo area: Set of typical data

| Water treatment plants | Date 1987 | Sample replicate | MPN/100 mL Total coliform | Fecal coliform EC | Fecal coliform A-1 | MFC fecal/coliform/ 100 mL | Coliphage* PFU/100 ml |
|---|---|---|---|---|---|---|---|
| TRG | 01/06 | A | | 2 | 3 | 1 | |
| | | | 1300 | 50 | 70 | 42 | <5 |
| | | B | | 2.5 | 2.5 | 1 | |
| | | | 500 | 90 | 90 | 46 | <5 |
| | | C | | 2 | 3 | 1 | |
| | | | 1300 | 70 | 90 | 55 | <5 |
| TRC | 01/09 | A | | 3 | 1 | 2 | |
| | | | 220 | 130 | 70 | 87 | <5 |
| | | B | | 3 | 2 | 1 | |
| | | | 280 | 220 | 170 | 84 | <5 |
| | | C | | 3 | 1 | 2 | |
| | | | 220 | 130 | 80 | 86 | <5 |
| TGT | 01/13 | A | | 2.5 | 2.5 | 1 | |
| | | | ≥16,000 | 9000 | 9000 | 3570 | 260 |
| | | B | | 3 | 2 | 1 | |
| | | | 16,000 | 9000 | 5000 | 2510 | 250 |
| | | C | | 2.5 | 2.5 | 1 | |
| | | | 16,000 | 5000 | 5000 | 1720 | 230 |
| TGU | 01/14 | A | | 2 | 3 | 1 | |
| | | | 140 | 80 | 110 | 28 | 10 |
| | | B | | 2 | 3 | 1 | |
| | | | 220 | 110 | 130 | 19 | 5 |
| | | C | | 2.5 | 2.5 | 1 | |
| | | | 220 | 80 | 80 | 25 | <5 |
| TCA | 01/07 | A | | 2.5 | 2.5 | 1 | |
| | | | 34 | 11 | 11 | 10 | <5 |
| | | B | | 1.5 | 1.5 | 3 | |
| | | | 70 | 17 | 17 | 26 | <5 |
| | | C | | 1 | 2 | 3 | |
| | | | 30 | 7 | 14 | 18 | <5 |
| TCB | 01/08 | A | | 3 | 1 | 2 | |
| | | | 16,000 | 2400 | 1300 | 1600 | 755 |
| | | B | | 3 | 2 | 1 | |
| | | | 16,000 | 2200 | 800 | 750 | 510 |
| | | C | | 3 | 1 | 2 | |
| | | | ≥16,000 | 3000 | 800 | 1350 | 620 |

* PFU: plague-forming units.

are given in Table III. These results indicate a strong positive correlation among the coliform/fecal coliform tests, while the correlations of the coliphage test with these tests are not as strong but appear to be nearly constant. The above correlations reflect the



TABLE II
Summary statistics for the microbiological data in raw water

| Parameter | Mean $\bar{r}$ | Standard deviation S | Coefficient of variation S/F | Median | Minimum | Maximu |
|---|---|---|---|---|---|---|
| Total coliform (TC) | 8929 | 30,152 | 3.377 | 500 | <2 | 160,00 |
| Fecal coliform (EC) | 1654 | 6351 | 3.840 | 80 | <2 | 50,00 |
| Fecal coliform (A1) | 1735 | 6484 | 3.737 | 70 | <2 | 50,00 |
| Fecal coliform (M-FC) | 909 | 2522 | 2.774 | 70 | <1 | 21,00 |
| Coliphage | 221.7 | 608.9 | 2.747 | 5.0 | <5 | 40[illegible] |

associations between the pairs of tests as a result of time and site variability. It would be interesting to compute the correlation matrix for each location.

Table IV presents results indicating that there are substantial differences between the correlations from location to location. For example, the correlations between the coliphage and total coliform and fecal coliform tests are negative for location TRC, while the same correlations are positive and quite large for site TGU. The reason for this is due to the degree of variability within each location. This can be seen from Table V, which gives the $F$ ratios that resulted from performing a one-way analysis of variance. It can be seen that the locations with low correlations between coliphage and each coliform/fecal coliform test have the lowest $F$ ratio. Furthermore, the coliphage test (except for the TGU location) has the lowest variance ratio, which means it has the lowest variability over time.

The second issue to be considered is a comparison of the fecal coliform counts associated with the EC MPN, A-1 MPN, and M-FC population estimation techniques. The analysis in Table III shows the existence of positive and significant correlations between these techniques, which indicates that applications of these techniques have produced similar patterns. However, this is not an indication for the equivalence of the results of the three tests. The Friedman's rank sums

TABLE III
Spearman's rank correlation matrix: Raw water

| | TC | EC | A-1 | M-FC |
|---|---|---|---|---|
| EC | 0.84 | | | |
| A-1 | 0.87 | 0.93 | | |
| M-FC | 0.85 | 0.90 | 0.93 | |
| Coliphage | 0.74 | 0.77 | 0.78 | 0.76 |

TABLE IV
Spearman's rank correlation for data within each location: Raw water

| | EC | A-1 | M-FC | Coliphage | EC | A-1 | M-FC | Coliphage |
|---|---|---|---|---|---|---|---|---|
| | | TRG | | | | | TRC | |
| TC | 0.421 | 0.570 | 0.651 | 0.111 | 0.63 | 0.63 | −0.02 | −0.22 |
| EC | | 0.852 | 0.441 | 0.480 | | 0.75 | 0.18 | −0.14 |
| A-1 | | | 0.621 | 0.275 | | | 0.48 | −0.37 |
| M-FC | | | | 0.300 | | | | −0.38 |
| | | TGT | | | | | TGU | |
| TC | 0.74 | 0.84 | 0.33 | 0.50 | 0.39 | 0.48 | 0.25 | 0.75 |
| EC | | 0.78 | 0.51 | 0.63 | | 0.51 | 0.50 | 0.55 |
| A-1 | | | 0.09 | 0.51 | | | −0.12 | 0.71 |
| M-FC | | | | 0.48 | | | | 0.25 |
| | | TCA | | | | | TCB | |
| TC | 0.03 | 0.08 | 0.27 | −0.23 | 0.86 | 0.87 | 0.48 | 0.09 |
| EC | | 0.74 | 0.46 | 0.11 | | 0.92 | 0.59 | 0.23 |
| A-1 | | | 0.39 | −0.13 | | | 0.55 | 0.24 |
| M-FC | | | | 0.21 | | | | 0.01 |

test (Hollander and Wolfe, 1973) was used to compare the three techniques. The sum ranks are shown in Table VI. The data in this table show that there are little differences among the sum ranks of the three techniques. These differences are not significant at the 1% level and are in agreement with the other studies in the IDRC coliphage program (Ratto *et al.*, 1988; Castillo *et al.*, 1988; El Abagy *et al.*, 1988).

## Drinking Water

Table VII presents a typical set of drinking water data used in the statistical analyses. The associations between the P/A, MF-Endo total

TABLE V
Variance *F*-ratios for the differences between times within each location

| | Bacteriological parameters | | | | |
|---|---|---|---|---|---|
| Locations | TC | EC | A-1 | M-FC | Coliphage |
| TRG | 54.04 | 25.42 | 45.32 | 32.97 | 3.43 |
| TRC | 15.12 | 40.93 | 47.21 | 67.09 | 0.79 |
| TGT | 78.32 | 98.81 | 149.34 | 96.44 | 34.83 |
| TGU | 48.95 | 37.61 | 24.25 | 76.20 | 40.43 |
| TCA | 60.47 | 17.10 | 11.70 | 27.46 | 2.12 |
| TCB | 100.13 | 143.61 | 79.78 | 43.81 | 41.80 |



TABLE VI
Sum ranges for EC, A-1, and M-FC

| | Fecal coliform method | | |
|---|---|---|---|
| | EC | A-1 | M-FC |
| Sum rank | 262 | 252.5 | 265 |

coliform and $H_2S$ paper strip tests are displayed in Table VIII. Each contingency table gives the number of samples where both tests are positive or negative, and the number of samples with one test positive and the other negative. To test the significance of the observed association, the McNemar (Lehman, 1975) test was applied to each pair of bacteriological tests and the results are shown in Table IX. There are significant ($p < 0.01$) differences between P/A and both $H_2S$ and total coliform (TC) tests while the difference between $H_2S$ and TC is not significant. The P/A test produced more significant positive results than the other two tests (a finding also supported by the studies of El Abagy *et al.*, 1988, Castill *et al.*, 1988, and Ratto *et al.*, 1988).

The ability of the P/A test to identify the presence of coliforms was compared to that of the total coliform MF Endo test. The McNemar test is significant at the 1% level which indicates that the TC MF technique was more effective in detecting the presence of coliforms than the P/A test. The summary of the association data is given in Table X.

Only one sample, No. 110 bottled potable water, was found to contain coliphage 5 PFU/100 mL. The P/A test was the only bacteriological test positive in this sample, and the bacteria contaminating this water sample were *Aeromonas* species.

## CONCLUSIONS

In this study, the coliphage test when applied to raw water samples showed the least correlations with the other microbiological tests. This indicates that coliphage is either a less sensitive test of fecal pollution in comparison to the other tests, or that coliphage is an indication of other types of pollution. There were no significant differences between the EC, A-1 and MFC fecal coliform techniques.

In the potable water samples, coliphage were only found in one sample, a bottled water sample, an indication of possible sanitation technique failure.

The superiority of the sensitivity of the P/A test is readily shown in the potable water study. The P/A test is relatively inexpensive compared to membrane filter and traditional TC/FC MPN procedures,

TABLE VII

Microbiological results—Potable water samples; Set of typical data, 1986[a]

| | | | | P/A test | | | | | | | $H_2S$ test | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | + or − | | | Bacteria identified | | | | |
| Sample number | Water source | Month/day | Free residual chlorine (mg/L) | TC | FC | FS | Cl.P | P.A | S.A | Aerom | 22°C | 30°C | 35°C | 22°C | 30°C | 35°C | Coliphage PFU/100 mL | TC MF-Endo /100 mL |
| 97 | Well | 10/15 | — | P | P | P | A | P | A | A | +++ 36 h | +++ 24 h | ++ 24 h | C.F. | C.F. | C.F. | <5 | 25 |
| 98 | Well | 10/15 | — | P | P | P | A | A | A | A | + 24 h | +++ 18 h | +++ 18 h | C.F. | C.F. | C.F. | <5 | 3440 |
| 99 | Well | 10/15 | — | P | P | P | A | A | A | A | +++ 36 h | +++ 24 h | + 24 h | C.F. | C.F. | C.F. | <5 | 1200 |
| 100 | Well | 10/15 | — | P | P | P | A | A | A | A | +++ 36 h | + 18 h | +− 18 h | C.F. | C.F. | C.F. | <5 | Confluent |
| 101 | Bottled water | 10/15 | — | A | A | A | A | A | P | A | — | — | — | | | | <5 | Confluent |
| 102 | Bottled water | 10/15 | — | A | A | A | A | A | P | A | — | — | — | | | | <5 | <1 |
| 103 | Bottled water | 10/15 | — | P | A | A | A | A | A | A | — | — | — | | | | <5 | 1 |
| 104 | Bottled water | 10/15 | — | A | A | A | A | P | A | A | — | — | — | | | | <5 | <1 |
| 105 | Bottled water | 10/15 | — | A | A | A | A | A | P | A | — | — | — | | | | <5 | 9 |
| 106 | Bottled water | 10/15 | — | P | P | A | A | A | A | A | — | — | — | | | | <5 | Confluent |
| 107 | Bottled water | 10/15 | — | A | A | A | A | P | A | A | — | — | — | | | | <5 | <1 |
| 108 | Bottled water | 10/30 | — | A | A | A | A | P | A | A | — | ++ 36 h | ++ 36 h | — | P.A | P.A | <5 | <1 |
| 109 | Bottled water | 10/30 | — | A | A | A | A | A | A | P | — | — | — | | | | 5 | <1 |
| 110 | Bottled water | 10/30 | — | A | A | A | A | A | A | P | — | — | — | | | | <5 | <1 |
| 111 | Bottled water | 10/30 | — | A | A | A | A | A | A | P | — | — | — | | | | <5 | <1 |
| 112 | Bottled water | 10/30 | — | A | A | A | A | A | A | A | — | — | — | | | | <5 | <1 |

[a] TC, total coliform; FC, fecal coliform; FS, fecal streptococci; Cl.P, *Clostridium perfringens*; P.A, *Pseudomonas aeruginosa*; S.A., *Staphylococcus aureus*. Aerom, *Aeromonas*. C.F., *Citrobacter freundii*.

TABLE VIII

Contingency tables for the association between bacteriological methods

| | | $H_2S$ − | $H_2S$ + | | | | TC − | TC + | | | | $H_2S$ − | $H_2S$ + | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P/A | − | 85 | 3 | 88 | P/A | − | 86 | 2 | 88 | TC | − | 95 | 4 | 99 |
| | + | 16 | 8 | 24 | | + | 13 | 11 | 24 | | + | 16 | 7 | 13 |
| | | 101 | 11 | 112 | | | 99 | 13 | 112 | | | 101 | 11 | 112 |

TABLE IX

McNemar test for comparing bacteriological methods in drinking water

| | $H_2S$ | TC |
|---|---|---|
| P/A | 2.98[a] | 2.84[a] |
| $H_2S$ | | 0.633 |

TABLE X

A contingency table for comparing the P/A and TC tests in detecting the presence of coliforms

| | | TC − | TC + | |
|---|---|---|---|---|
| P/A | − | 99 | 4 | 103 |
| | + | 0 | 9 | 9 |
| | | 99 | 13 | 112 |

is simple to perform, and the authors recommend the P/A test without reservation for all routine potable water quality analyses. The P/A procedure combined with the $H_2S$ paper strip technique are both very amenable for use in routine laboratories and in remote field laboratories.

The $H_2S$ paper strip technique has been shown to be equally sensitive to the MF-Endo total coliform test for indicating the safety of potable water. Furthermore, the $H_2S$ technique is probably the best and simplest technique for testing remote water supplies.

The authors believe the P/A and $H_2S$ procedures combined with the coliphage test would provide an excellent assessment of the safety of potable waters from bacterial and virus contamination.

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