Coliphage Association with Coliform Indicators: A Case Study in Peru

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Abstract

The associations between traditional coliform/fecal coliform indicator tests and coliphage, Presence/Absence (P/A), A-1 broth, and H_2S paper strip tests are evaluated for Peruvian waters.

Drinking water samples showed that the P/A test was the most sensitive, producing the greatest number of positive results. In drinking water, in some of the samples, the only indicator organisms present were coliphage. The incidence of coliphage in these potable water supplies reflects the probability of human pathogenic viruses also surviving the treatment processes accorded those samples.

INTRODUCTION

The developing countries have been trying to supply their people with adequate supplies of safe drinking water as part of their commitment to the United Nations Program, the International Drinking Water and Sanitation Decade. These water supply program goals are often extremely difficult to achieve in developing countries and sometimes in the developed countries. This difficulty is mainly due to limited resources, both financial and technical, which must be split among many priorities.

In Peru, water quality standards are based on the coliform test and these standards are supported by legislation, Peruvian Law No. 1705. This law authorizes the Division of the Environment, Ministry of Health (DIGEMA), to assume responsibility for water quality surveillance, and to formulate and revise technical standards for the control of drinking water quality. However, the health service and water authority laboratories in Peru are poorly organized and equipped. Even

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though the Ministry of Health does operate a basic centralized laboratory service within DIGEMA, it is very rare, even within the capital city of Peru, Lima, to find potable water from distribution systems that is bacteriologically safe. Thus, the majority of the Peruvian people depend on bottled or boiled tap water due to the inavailability of state agencies to properly monitor and treat water supplies.

An important factor in the development and maintenance of a safe water supply is the ability to assess quickly and economically the microbiological quality of the potable water and their sources.

A research study was undertaken, through the financial and consultant support provided by the International Development Research Centre (IDRC), Ottawa, Canada, to evaluate the use of coliphage as an indicator of the sanitary quality of the source water for potable water supplies. The final goal of this research was to develop a classification system for the potable water source based on coliphage counts and sanitary services of the sites.

Combined with the above study was another research project that was based on the comparison and evaluation of a variety of microbiological water quality assessment techniques for use with potable waters. These tests, some of which were used routinely and experimentally in Peru, were evaluated for their sensitivity in Peruvian waters, their cost, and their dependence on costly important supplies and equipment. Another very important feature of this evaluation scheme was the criterion of whether these tests could be carried out in understaffed and underequipped rural laboratories.

The results of these studies are presented.

METHODS

Water Samples

Raw drinking water samples were collected in triplicate from the following sources: Rimac River, Santa Eulalia River, Chillon River, Lurin River, Mala River, and groundwater and springs in rural areas.

The following potable water samples were collected for microbiological testing: (a) 25 samples each of bottled drinking water with gas and without gas, and (b) 54 chlorinated drinking water samples from wells and distribution systems within Lima, Peru.

Coliphage Tests

The procedure described by Wetsel *et al.* (1982) and reproduced in section 919C of the American Public Health Association (APHA) *Standard Methods* (1985), with the addition of 2,3,5-triphenyl tetrazolium chloride and using *E. coli* C (ATCC No. 13706) as host, was used in this study.

Escherichia coli strains frequently isolated from Peruvian waters—*E. coli* CLEIBA$_1$ and *E. coli* CLEIBA$_2$—and from Brazilian waters—*E. coli* 2262-4 and *E. coli* 28767-7—were compared to *E. coli* C (ATCC No. 13706) in a 27 water sample study for their sensitivity and selectivity as potential coliphage hosts for the Peruvian study.

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 (MF), fecal coliform procedure using M-FC agar and Gelman GN-6 0.45-μ membrane filter.

Hydrophobic square-gridded membrane filters developed by Sharpe (1981) and marketed as the ISO-GRID method (QA Laboratories, Toronto, Canada) were also used with M-FC agar to estimate fecal coliform populations.

Drinking water samples were subjected to the following APHA *Standard Methods* (1985) total and fecal coliform tests and heterotrophic plate count test: the five-tube MPN procedure using lauryl tryptose broth and brilliant green lactose bile broth with fecal coliform confirmation in EC broth; and the 35°C heterotrophic spread plate count procedure using tryptone glucose extract agar.

All drinking water samples were also tested by the Presence/Absence (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 as used in the P/A test.

Chemical Tests

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

Biochemical Identification of Fecal Coliforms

A selected number of samples positive for fecal coliforms were subjected to isolation and identification procedures. Usually the last series of positive MPN tubes (A-1 broth) were streaked out onto MacConkey agar and the predominant colonies identified. For the MF fecal coliform test, three or four countable colonies per plate were selected for identification purposes. Isolated purified colonies were subjected to the following tests: oxidase, IMVIC, ornithine and lysine decarboxylase, and growth in Kligler agar.

Statistical Methods

Several nonparameteric statistical methods were applied to evaluate the association among bacteriological tests, which can be found in Hollander and Wolfe (1973).

RESULTS AND DISCUSSION

With no background data on the specificity of South American strains of *E. coli* to act as coliphage hosts, it was decided to evaluate and compare several commonly isolated South American strains of *E. coli* for their ability to act as universal hosts. The four *E. coli* strains selected—$CLEIBA_1$ and $CLEIBA_2$ (Peru) and 2262-4 and 28767-7 (Brazil)—were evaluated in several different natural waters. The *E. coli* strains 2262-4 and 28767-4 were only evaluated in two water samples as these samples produced coliphage plaques of 4960 and 2185 per 100 mL with the *E. coli* C and no plaques with hosts 2262-4 and 28767-4.

The CLEIBA *E. coli* host strain produced a mean plaque count of 1882 compared to a mean plaque count of 5722 for the *E. coli* C host in Rimac River and well water samples (1) during the period 10-8-86 to 29-8-86. Water samples tested using the $CLEIBA_2$ *E. coli* host produced a mean coliphage plaque count of 2095 compared to 2130 for the *E. coli* C host (16 samples). However, when only September data are compared, mean plaque count for $CLEIBA_2$ was 2293 compared to the *E. coli* C mean count of *1005*. The difference between these counts was due to a single sample collected from the Rimac River on 25-9-86 which produced 750 plaques on *E. coli* C and 9500 plaques on $CLEIBA_2$. However, based on the overall results from all comparisons and the recommendations of APHA (1985), ASTM (1982), and the work of Wetzel *et al.* (1982), it was decided to continue the rest of the research using *E. coli* C as the host strain.

RAW WATER

A total of 16 triplicate water samples were collected as part of this study. Representative samples of the data obtained from these waters are shown in Table I. One of the striking features of these data is the great consistency, at times, between replicates and, at other times, the great dissimilarity between replicates.

Isolates collected from positive A-1 broth tubes were all confirmed as *E. coli*. Similarly, all the countable colonies isolated and identified from M-FC agar proved to be *E. coli*. In both instances, the techniques were 100% selective for *E. coli* in the waters sampled.

Figure 1 gives the box plots of various microbiological data (log scale). The plots show that the log transformation does not only provide a convenient scale for comparing the results of several microbiological techniques, but it also yields symmetric distributions for the total and fecal coliform data. The plots show that total coliform data have the least spread while that of the coliphage have the highest spread. From the plots, the fecal coliform tests can be grouped into two groups, with the tests within each group being similar. One group consists of the EC and A-1 MPN broth tests, and the other includes the Gelman membrane filter and ISO-GRID MF tests.

Table II gives the Spearman's rank correlation matrix for the bacteriological techniques and temperature. The results indicate a negative but statistically insignificant association between bacteriological

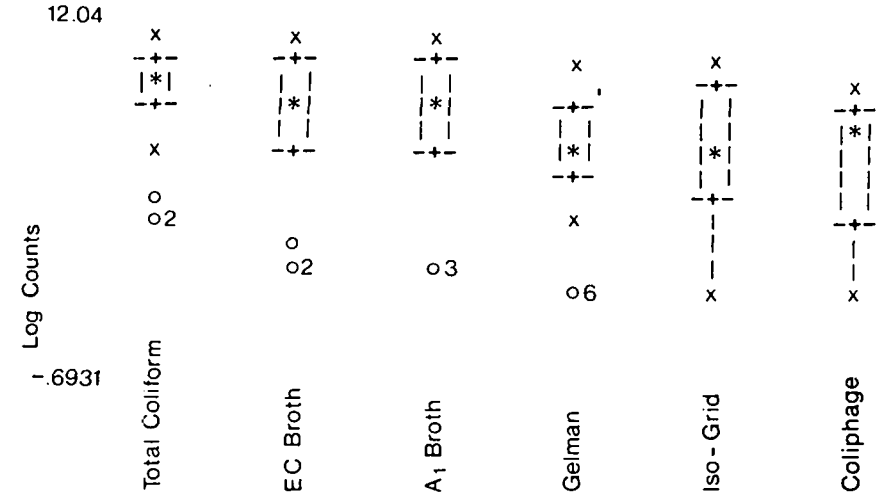

Fig. 1. Box plots for the distribution of bacteriological data in raw water.

TABLE I
Selected examples of raw water illustrating microbiological and coliphage patterns in replicate data

Sampling site	Replicate	Total coliform MPN/100 L	Fecal coliform/100 mL				Coliphages PFU*/100 mL
			EC broth MPN	A-1 broth MPN	M-FC agar Gelman MF	ISO-GRID MF	
Rimac River	1	160,000	160,000	50,000	25,000	40,000	15,350
20 km	2	90,000	50,000	50,000	16,000	27,000	13,850
	3	160,000	160,000	50,000	33,000	34,000	15,900
	Mean	136,000	136,000	50,000	24,600	33,600	15,033
Well	1	26	<2	<2	0	0	0
	2	30	<2	<2	0	0	0
	3	23	4	<2	0	0	0
	Mean	26.3	1.3	<2	0	0	0
Well	1	16,000	5000	1300	3000	3000	1040
SMP	2	9000	3000	3000	3000	2600	1160
	3	9000	9000	2400	1600	3000	1530
	Mean	11,300	5600	2230	2530	2866	1243
Lurin River	1	2400	300	500	340	300	0
	2	1700	700	300	190	70	10
	3	800	500	300	20	50	5
	Mean	1633	500	366	183	140	5
Lurin River	1	30,000	8000	8000	1300	800	3500
5 km	2	23,000	8000	4000	800	800	3500
	3	30,000	4000	8000	700	700	6000
	Mean	27,666	5333	5333	933	833	4333

* PFU: plaque-forming units.

TABLE II
Spearman's rank correlation matrix

		°C[a] C8	TC[b] C9	EC C10	A-1 C11	Gelman MF C12	ISO-GRID MF C13
TC	C9	−0.132					
EC	C10	−0.085	0.944[c]				
A-1	C11	−0.101	0.839[c]	0.901[c]			
Gelman	C12	−0.023	0.629[c]	0.654[c]	0.594[c]		
ISO-GRID	C13	−0.058	0.636[c]	0.670[c]	0.601[c]	0.977[c]	
Coliphage	C14	−0.257	0.856[c]	0.888[c]	0.908[c]	0.566[c]	0.566[c]

[a] °C: temperature.
[b] TC: total coliforms.
[c] Correlations are significant at the 1% level.

tests and temperature. All bacteriological tests are positively and highly ($p < 0.01$) correlated. The correlation values indicate that the membrane filter and ISO-GRID tests produced similar results, and the same is true for the EC and A-1 tests. The results of the coliphage test have correlations exceeding 0.85 with total coliform, EC, and A-1, while their correlations with the membrane filter and ISO-GRID tests are less than 0.6.

Fecal coliform tests were further evaluated using Freedman's rank sum test for the two-way layout with water samples representing the level of the first factor and the four fecal coliform tests representing the levels of the second factor. The observed value of the test is 76.544, which is highly significant ($p < 0.01$) when compared to the critical values of the chi-square distribution on 3 degrees of freedom. The sum of the ranks associated with each test is given in Table III, which shows that the EC MPN broth test produced the highest estimate of the fecal coliform population and the Gelman membrane filter procedure produced the lowest estimate of the population. To evaluate these differences further, Table IV gives the absolute differences in the sum of the ranks for each pair of tests and the results of performing the

TABLE III
Sum ranks associated with each fecal coliform test

	Fecal coliform test			
	EC MPN	A-1 MPN	Gelman MF	ISO-GRID MF
Sum ranks	139.5	123.0	58	69.5

TABLE IV
Absolute differences between the sum ranks of each pair of fecal coliform tests

	Fecal coliform test			
	EC MPN	A-1 MPN	Gelman MF	ISO-GRID MF
Fecal coliform tests				
EC		16.5	81.5[a]	70.0[a]
A-1			65.0[a]	53.5[a]
Gelman				11.5
ISO-GRID				

[a] Values are significant at the 1% level.

multiple comparison tests, which are associated with the Freedman's test. The results show that there are no significant differences between the EC and A-1 techniques, or between the Gelman membrane filter and ISO-GRID membrane filter tests. The tests can be classified into two groups: the first consists of EC and A-1, and the second with the two membrane filtration procedures.

Drinking Water

Fifty samples of bottled drinking water were examined, 25 with carbonation (gas) and 25 without carbonation (no gas) for presence of indicator bacteria and heterotrophic bacterial densities. Table V presents data from the bottled potable waters (8 with gas and 14, no gas) testing positive by one of the bacterial indicator techniques. Thirty-two percent of the carbonated bottled waters contained microbial contaminants, 28% contained *P. aeruginosa,* and 20% contained total coliforms. Bottled waters without carbonation showed that 56% of the samples were positive for microbial contaminants, 40% contained *P. aeruginosa* and 32% contained total coliforms. Eight of the bottled water samples were found to contain total coliforms by the P/A test but were negative for total coliforms by the traditional Peruvian MPN TC test.

Of the 54 potable water samples collected from distribution lines and wells that are subject to chlorination, 14 samples (Table VI) (25.9%) were positive for contaminating bacteria, 9.3% shown by the P/A test alone, 5.5% shown by the P/A and TC MPN test, and 1.85% shown by the H_2S paper strip test alone.

Figure 2 displays the set of contingency tables that summarize the

Bottled Water with Gas

P/A \ H_2S	−	+	
−	17	0	17
+	8	0	8
	25	0	25

Bottled Water without Gas

P/A \ H_2S	−	+	
−	11	0	11
+	9	0	14
	20	0	25

Distribution System I

P/A \ H_2S	−	+	
−	32	1	33
+	8	1	9
	40	2	42

Distribution System II

P/A \ H_2S	−	+	
−	10	1	11
+	0	9	9
	10	10	20

Distribution Systems I & II

P/A \ H_2S	−	+	
−	42	2	44
+	8	10	18
	50	12	62

Wells

P/A \ H_2S	−	+	
−	9	0	9
+	4	0	4
	13	0	13

All Data

P/A \ H_2S	−	+	
−	79	2	81
+	29	15	44
	108	17	125

Fig. 2. Contingency tables for P/A and H_2S in different drinking water types.

TABLE V

Bottled water samples, positive by one or more bacterial indicator tests

Water source	Free residual chlorine (mg/L)	Fecal coliform							H_2S test/100 mL + or neg		Bacteria	Isolated	MPN/100 mL		HPC[h]
		TC[a]	FC[b]	FS[c]	Cl[d]	P.a[e]	S.a[f]	Aer[g]	22°C	35°C	22°C	35°C	TC	FC	/mL
Bottled water with gas	–	A	A	A	A	P	A	P	–	–			<2	<2	5100
Bottled water with gas	–	A	A	A	A	P	A	A	–	–			<2	<2	6200
Bottled water with gas	–	A	A	A	A	P	A	A	–	–			<2	<2	6500
Bottled water with gas	–	P	P	A	A	P	A	A	–	–			<2	<2	30
Bottled water with gas	–	P	P	A	A	P	A	A	–	–			<2	<2	50
Bottled water with gas	–	P	P	A	A	P	A	A	–	–			<2	<2	68
Bottled water with gas	–	P	P	A	A	P	A	A	–	–			<2	<2	1520
Bottled water with gas	–	P	P	A	A	P	A	A	–	–			<2	<2	1480
Bottled water no gas	–	P	A	A	A	A	A	A	+	+	*Citrobacter* *Klebsiella*	*Citrobacter* *Klebsiella*	<2	<2	112
Bottled water no gas	–	P	A	A	A	A	A	A	–	–			<2	<2	105
Bottled water no gas	–	P	P	P	A	P	A	A	+	+	*E. coli* *Pseudomonas*	*Citrobacter* *E. coli* *Pseudomonas*	12	7	2140
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	520
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	350
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	2200
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	295
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	16000
Bottled water no gas	–	P	A	A	A	A	A	A	–	–			<2	<2	1300
Bottled water no gas	–	P	P	A	A	A	A	A	–	–			<2	<2	2400
Bottled water no gas	–	A	A	A	A	P	A	A	–	–			<2	<2	3600
Bottled water no gas	–	P	A	A	A	P	A	A	+	+	*Citrobacter*	*Citrobacter*	26	<2	9500
Bottled water no gas	–	P	A	A	A	P	A	A	+	+	*Citrobacter*	*Citrobacter*	22	<2	1200
Bottled water no gas	–	P	A	A	A	P	A	A	+	+	*Citrobacter*	*Citrobacter*	17	<2	521

[a] TC: total coliforms.
[b] FC: fecal coliforms.
[c] FS: fecal streptococci.
[d] Cl: Clostridium.
[e] P.a: *P. aeruginosa*.
[f] S.a: *S. aureus*.
[g] Aer: *Aeromonas*.
[h] HPC: heterotrophic plate count.

TABLE VI

Potable water samples, positive by one or more bacterial indicator tests[a]

Water source	Free residual chlorine (mg/L)	Fecal coliform							H_2S test/100 mL + or neg		Bacteria	Isolated	MPN/100 mL		HPC
		TC	FC	FS	Cl	P.a	S.a	Aer	22°C	35°C	22°C	35°C	TC	FC	/mL
Distribution system	0.10	P	P	A	A	A	A	A	–				<2	<2	210
Distribution system	0.25	P	P	A	A	A	A	P	+	+	*Aeromonas*	*Citrobacter* *P. aeruginosa* *Aeromonas*			200
Distribution system	0.0	P	P	A	A	P	A	A	–	–			<2	<2	298
Distribution system	0.0	P	P	A	A	P	A	A	–	–			<2	<2	195
Distribution system	0.1	A	P	A	A	P	A	A	–	–			<2	<2	6200
Distribution system	0.1	A	P	A	A	P	A	A	–	–			<2	<2	7200
Well	0.0	P	P	A	A	A	A	A	–	–			130	<2	3500
Distribution system	0.0	P	A	A	A	P	A	A	–	–			<2	<2	298
Well	0.0	P	A	A	A	A	A	A	–	–			130	<2	3500
Distribution system	0.0	P	A	A	A	P	A	A	–	–			<2	<2	298
Well	0.0	P	P	A	A	A	A	A	–	–			>1600	1600	8500
Well	0.1	P	A	A	A	A	A	A	–	–			<2	<2	11
Distribution system	0.25	P	A	A	A	P	A	A	–	–			<2	<2	13
Distribution system	0.25	A	A	A	A	A	A	A	+	+	*Citrobacter*	*Citrobacter* *Clostridium*	<2	<2	11

[a] See Table V for abbreviations.

information available about P/A and H_2S tests and their association for different types of drinking water. These tables show that the P/A test is more likely to produce positive results than the H_2S test and this appears to be consistent for all drinking water types. For example, the contingency table for all the data show that out of 125 samples, 44 were positive on the basis of the P/A test and only 17 samples were positive using the H_2S test. Although the above comparison is useful, it can lead to the incorrect evaluation of the sensitivity of the tests. The proper way of comparing the two tests is to restrict the statistical analysis to the cells of the contingency table corresponding to + – and – +. The statistical test statistic for doing this is the McNemar statistic. Table VII gives the observed values of this test. The results show that the P/A test produces more significant positive results than H_2S test.

Figure 3 gives the box plots for the ln HPC and ln TC for different entries to the contingency tables when there are sufficient data for representing the box plot. For bottled waters the median log HPC is 7.3 when the P/A test is positive and the H_2S is negative. This can be compared to the median of 4.1 when both tests are negative. One major feature is that the box plot corresponding – + class shows more spread than that corresponding to – – class. Similar conclusions can be reached for Distribution System I and for the data from the wells.

The box plots for total coliforms, on the other hand, are significantly different in the case of the class + + from all the other classes. This perhaps indicates that when both tests are positive they are indicative of the presence of coliform bacteria.

The contingency tables (Fig. 4) show the association between the P/A test and the fecal and total coliform MPN tests. These tables show that a positive and or negative result using the MPN test is always associated with a positive and or negative result for the P/A test, but the P/A test is more able to detect the presence of fecal and total coliform in the water samples than the MPN tests. McNemar statistics for comparing the P/A test with the FC and TC tests are 3.741 and

TABLE VII

McNemar statistics for comparing P/A with H_2S

	Bottled water		Distribution systems			Wells	All data
	Gas	No gas	I	II	III		
McNemar statistic	2.826[b]	3[b]	2.333[a]	1	1.896	2_{10}[a]	4.85[b]

[a] Significant at the 5% level.

[b] Significant at the 19% level.

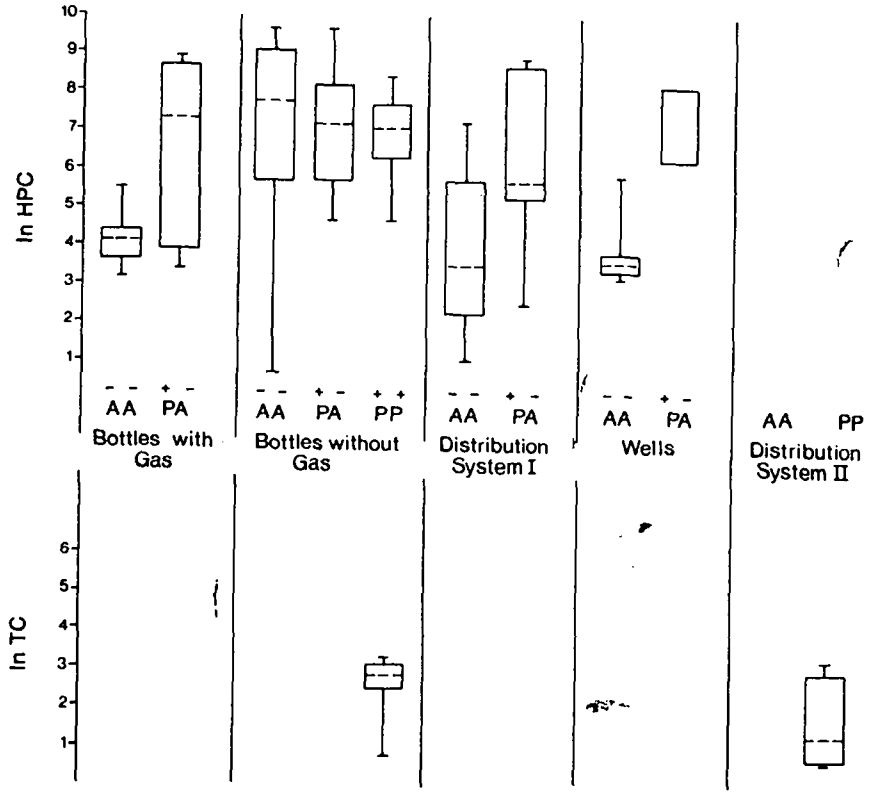

Fig. 3. The association between P/A, H_2S and HPC and TC.

		FC H_2S −	+	
P/A	−	99	0	99
	+	14	11	25
		113	11	124

		TC H_2S −	+	
P/A	−	91	0	91
	+	18	15	33
		109	15	124

Fig. 4. Contingency tables for the P/A test and the MPN tests for TC and FC.

4.243, respectively, which are significant at the 1% level. This provides strong evidence for the superiority of the P/A test when compared to the FC and TC tests in detecting the presence of coliforms in the water.

In these samples the H_2S paper strip technique proved to be more sensitive than the Peruvian MPN TC test for indicating the presence of contaminating bacteria. From these data, it can be concluded that the P/A test is the most sensitive procedure for indicating the presence of contaminating bacteria. Also, the P/A test has been found to be less costly (materials and manpower) than traditional Peruvian bacteriological water quality testing procedures.

The H_2S paper strip method also continues to show that it produces results very similar to traditional Peruvian TC MPN drinking water testing procedures at a fraction of the cost. Of all the procedures evaluated, the H_2S test is the simplest to perform and is the least costly (materials and manpower). Based on these bottled water and potable water studies, it appears to be equally safe/hazardous to drink bottled water with or without gas and water from Lima potable water distribution systems.

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