Wat. Res. Vol. 23, No. 2, pp. 253–255, 1989
Printed in Great Britain.

0043-1354/89 $3.00 + 0.00

POTABLE WATER SAFETY ASSESSED BY COLIPHAGE AND BACTERIAL TESTS

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(*First received May 1988; accepted in revised form October 1988*)

Abstract—Twenty samples of drinking water from five different distribution line sources in Lima, Peru, were tested for coliphage content. Bacteriological quality of these waters was assessed by a variety of techniques, Presence/Absence (P/A) test, H_2S paper strip test and total and fecal coliform MPN tests. In 47% of the samples, coliphage were the only indicator organisms present. The incidence of coliphage in these potable water supplies reflects the probability of human pathogenic virus also surviving the treatment process. In the bacteriological tests the P/A and H_2S paper strip techniques were found to be equally or more sensitive indicators of the presence of coliform/fecal coliform bacteria than the TC/FC MPN tests were.

Key words—coliphage, potable water, P/A test, H_2S tests, developing country

INTRODUCTION

This report is part of a three continent, eight country study, sponsored by the International Development Research Centre (IDRC), Ottawa, Canada, to investigate the potential use of coliphage counts to categorize raw drinking water sources. The goal of this IDRC study is to select one or more microbiological tests that are simple, reliable and can be carried out by nominally trained personnel under minimal laboratory conditions.

One part of this study was also to evaluate potable water supplies, both bottled and tap, using coliphage counts in addition to the routine bacteriological tests of the country involved. Data from these studies indicated the presence of coliphage in coliform-free tap and bottled potable waters (Sim and Dutka, 1987; El-Abagy *et al.*, 1988).

In this report we present the results of a study in Peru on water samples collected during October and November, 1987, from the City of Lima potable water distribution system. These results concur that the presence of coliphage in coliform-free potable waters is an international occurrence.

METHODS

Water samples

Twenty water samples were collected from five different taps in one distribution system over a 3 week period. Samples were dechlorinated with sodium thiosulphate (APHA, 1985) and maintained at ambient temperature until processing was completed, within a maximum of 4 h of collection.

Microbiological tests

Potable water samples were subjected to the APHA *Standard Methods* (1985) five tube most-probable-number technique for coliforms and fecal coliforms using laural tryptose broth and brilliant green lactose bile broth with fecal coliform confirmation in EC broth. The water samples were also tested by the P/A test (Clark, 1969) and all positive samples 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 H_2S paper strip technique (Manja *et al.*, 1982) using chemically inoculated paper strips incubated at 22 and 35°C was also used to test the water samples for contaminating bacteria. All positive samples were subjected to similar confirmation procedures as used in the P/A test (Clark *et al.*, 1982).

The coliphage procedure described by Wentsel *et al.* (1982) with the addition of 2,3,5-triphenyl tetrazolium chloride and using *E. coli* C (ATCC No. 13706) as host was also used to test the potable waters (APHA, 1985). The above procedure was made more sensitive by increasing the volume of water sample tested to enable measurement of 1 plaque forming unit per 100 ml sample.

Chemical tests

Free residual chlorine was assessed in all samples using amperometric titration procedures (APHA, 1985).

RESULTS AND DISCUSSION

Data obtained during this study are presented in Table 1. Fecal streptococci, *Staphylococcus* spp, *Aeromonas* spp, and *Clostridium perfringens* were not isolated in the P/A medium. Coliphage were found in 16 of the 20 samples, and 9 of these 16 contained no coliform organisms or other indicator bacteria. Conversely in only three samples (1, 3 and 4), positive for coliform bacteria, were coliphage not detected.

Only four water samples were found to contain detectable levels of residual chlorine, 0.1 mg/l. Three

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Table 1. Results of bacterial and coliphage tests on potable water samples collected from Lima, Peru potable water distribution lines

Sample number	Date	Free residual chlorine	P/A test/100 ml			H_2S production test				TC MPN /100 ml	FC MPN /100 ml	Coliphage PFU** /100 ml
						Pos. or neg.		Bacteria identified				
			TC*	FC†	*P.a.*¶	22°C	35°C	22°C	35°C			
1	19-10	0.0	P‡	P	A	+	+	Citrobacter	Citrobacter *E. coli*	<2	<2	0
2	19-10	0.0	P	P	A	+	+	Citrobacter	Citrobacter *E. coli*	4	4	1
3	19-10	0.0	P	P	A	−	+	Not confirmed	Citrobacter *E. coli*	12	9	0
4	19-10	0.0	P	P	A	+	+	Citrobacter	Citrobacter *E. coli*	2	2	0
5	19-10	0.0	P	P	A	−	+	Not confirmed	Citrobacter *E. coli*	21	13	13
6	26-10	0.0	A§	A	A	+	+	Not confirmed	Citrobacter *E. coli*	<2	<2	8
7	26-10	0.0	P	P	A	−	+		Citrobacter *E. coli*	4	<2	15
8	26-10	0.0	A	A	A	−	−			<2	<2	9
9	26-10	0.0	P	P	A	−	−			2	2	5
10	26-10	0.0	A	A	A	−	−			<2	<2	2
11	2-11	0.0	A	A	A	−	−			<2	<2	1
12	2-11	0.0	A	A	A	−	−			<2	<2	12
13	2-11	0.1	A	A	A	−	−			<2	<2	3
14	2-11	0.1	A	A	A	−	−			<2	<2	57
15	2-11	0.1	A	A	A	−	−			<2	<2	1
16	9-11	0.0	A	A	A	−	−			<2	<2	4
17	9-11	0.0	A	A	A	−	−			<2	<2	14
18	9-11	0.0	P	P	A	+	+	Citrobacter *E. coli*	Citrobacter *E. coli*	21	21	9
19	9-11	0.0	P	P	A	+	+	Citrobacter *E. coli*	Citrobacter *E. coli*	26	6	2
20	9-11	0.1	A	A	A	−	−			<2	<2	0

*Total coliforms. †Fecal coliforms. ‡Present. §Absent. ¶ *Pseudomonas aeruginosa*. **Plaque forming units.

of these contained only coliphage and the fourth sample (20) was free of coliphage, coliforms and other indicator bacteria.

In an extensive review of the early literature, Grabow (1968) strongly suggested that coliphage and the most common pathogenic viruses are much more resistant to chlorination treatment than are *E. coli*. Chambers (1971) and Havelaar (1986) have confirmed this view with studies on sewage treatment plant effluents. Thus the finding of coliphage in these drinking water samples, with and without coliform presence, suggests that viruses could also survive the treatment and disinfection process accorded these potable water samples (Havelaar, 1986).

Other implications of the data obtained from these studies are that coliform-free potable waters are not necessarily pathogen-free and chlorination practices in Lima potable water distribution systems are totally inadequate. The findings reported here concerning coliform-free but coliphage containing potable waters are not single rare events. Similar results have been reported from Singapore (Sim and Dutka, 1987) and Cairo (El-Abagy *et al.*, 1988) potable water supplies.

For every sample positive for indicator bacteria by the P/A test (Table 1), the H_2S paper strip test was also positive. The P/A test was positive in one sample in which the TC/FC MPN combination was negative and there were no samples where the TC/FC MPN tests were positive and the P/A and H_2S paper strip tests were negative. In one sample (6), the H_2S paper strip test was the only test positive for coliforms (*Citrobacter* spp and *E. coli*). The coliphage test was also positive in this sample.

These data are interesting as they indicate that the P/A and H_2S paper strip tests are equally or more sensitive for pollution indicator bacteria testing in potable water samples compared to traditional TC MPN procedures. Similar results were found in an earlier Egyptian study (El-Abagy *et al.*, 1988). Both the P/A and H_2S tests are extremely simple to carry out in routine and field laboratories even with minimally trained staff. These procedures are also much more cost effective than traditional TC/FC MF and MPN tests. In this study, the H_2S test appears to be equally sensitive at 22 and 35°C.

In summary, based on these and earlier studies (Sim and Dutka, 1987; El-Abagy *et al.*, 1988) we suggest that coliphage tests be included as part of any potable water testing scheme. The coliphage test has an advantage over traditional microbiological tests in that the test can be read after as little as 6 h, if necesary. It is very economical and simple to perform and its sensitivity can easily be increased by testing more 5 ml aliquots, by increasing the aliquot size to 25 or 50 ml or by using a coliphage MPN technique. The P/A and H_2S paper strip test results indicate that they are equally or more sensitive than TC MPN techniques in testing potable waters. Since both of these media can be prepared and maintained in sealed bottles for relatively long periods (4 months to 1

year), they would be ideal testing procedures for isolated water supplies and where laboratory facilities do not exist.

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