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ABSTRACT

Samples of drinking water from five different distribution line sources in Lima, Peru, were tested for total coliforms, fecal coliforms and coliphage populations. The P/A and H_2S paper strip methods were equally or more sensitive for the presence of indicator bacteria than the TC/FC MPN test. In 20% 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 virus presence.

RÉSUMÉ

Des échantillons d'eau potable ont été prélevés en cinq point du réseau de distribution de Lima, au Pérou, afin d'y dénombrer les coliformes totaux, les coliformes fécaux et les coliphages. Les papiers indicateurs de P/A et de H_2S étaient d'une sensibilité Equivalente ou supérieure à l'évaluation du M.P.N. de CT/CF quant à la présence de bactéries servant d'indicateurs. Dans 20 % des échantillons, les seuls organismes indicateurs qui ont été relevés étaient des coliphages. La présence de coliphages dans ces réseaux d'eau potable traduit la présence probable de virus pathogènes pour les humains.

MANAGEMENT PERSPECTIVE

Coliphages are viruses which infect E. coli and other fecal coliform bacteria. Ecotoxicology and Biomonitoring Project Team, NWRI has been evaluating and using coliphages as surrogate indicators of fecal pollution in receiving and drinking waters because the test is simple, inexpensive and, most importantly, the samples can be preserved for at least 72 hours before testing, thus opening up a greater portion of Canadian waters for biomonitoring.

Since coliphages are viruses, their reactions to disinfection, whether in the sewage treatment plant or drinking water treatment plant, are similar to other viruses. Thus the finding of coliphages in drinking water also implies that human pathogenic viruses can be present as the disinfection treatment was inadequate to remove coliphages.

The finding that the P/A and H_2S paper strip tests are equivalent to or more sensitive techniques (also less costly, simpler and require minimally trained staff to perform them) to traditional total coliform and fecal coliform tests applied to drinking water has important Canadian benefits. These results have important ramifications for the testing of potable water supplies in isolated northern settlements and Indian reserves.

The data for this report were obtained from an IDRC (International Development Research Centre, Ottawa) funded study in Peru, for which the co-author, B.J. Dutka was the study originator, consultant and report writer.

PERSPECTIVE-GESTION

Les coliphages sont des virus qui s'attaquent à E. coli et à d'autres bactéries coliformes fécales. L'équipe responsable du projet d'écotoxicologie et de surveillance biologique de l'INRE évalue et utilise les coliphages comme indicateurs de substitut de la pollution fécale dans les eaux réceptrices et les eaux potables. Ce test est simple et peu coûteux et, ce qui importe avant tout, les échantillons peuvent être conservés pendant au moins 72 heures avant d'être testés, ce qui permet d'envisager l'extension des programmes de surveillance biologique à un plus grand nombre de plans d'eau canadiens.

Étant donné que les coliphages sont des virus, leur réactions à la désinfection tant dans les stations d'épuration des eaux d'égouts que dans les installations de traitement des eaux potables sont similaires à celles des autres virus. Par conséquent, la présence de coliphages dans les eaux potables après la désinfection révèle que des virus pathogènes pour les humains peuvent également contaminer ces eaux.

Les papiers indicateurs de P/A et de H_2S sont d'une sensibilité équivalente ou supérieure aux techniques classiques de dénombrement des coliformes totaux et des coliformes fécaux dans l'eau potable (en outre, ces nouveaux tests sont moins coûteux et plus simples et ne requièrent qu'une formation minimale). Cette découverte est d'une grande importance au Canada, car nous pourrons ainsi envisager d'évaluer les réserves d'eau potable dans les localités isolées du nord et les réserves indiennes.

Les années sur lesquelles reposent ce rapport proviennent d'une étude réalisée au Pérou. L'étude, qui a été subventionnée par le CRDI (Centre de recherches pour le développement international), a été proposée par son coauteur, B.J. Dutka, qui a également servi d'expert-conseil et s'est chargé d'élaborer le rapport.

INTRODUCTION

This report is part of a three continent study, sponsored by the International Research Development Centre, 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 of the sub-studies built into this three continent, eight country study, was to also evaluate potable water supplies, both bottled and tap, using routine membrane filtration or most-probable-number bacteriological procedures of the country, plus one or all of the following tests, the P/A test[1], the H_2S paper strip test[2], ISO-Grid membrane filter technique[3] and coliphage counts[4]. Data from three of the countries involved in this international project have been reported elsewhere[5,6,7] and in the potable water studies one common factor was becoming evident and that was the repeated finding[6,7] of coliphage in bacteria free potable waters. In this report, we present the results of one minor study carried out on samples collected from the Lima potable water distribution system.

METHODS

Water Samples

Water samples were collected from five different taps in one distribution system over a four week period. Samples were dechlorinated prior to processing and processing was completed within a maximum of four hours of collection.

Microbiological Tests

Potable water samples were subjected to the APHA Standard Methods[8] 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[1] 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[9]. The H_2S paper strip technique[2] 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 identification procedures as used in the P/A test[9].

The coliphage procedure described by Wetsel[4] and reproduced in section 919C APHA Standard Methods[8] with the addition of 2,3,5-triphenyl tetrazolium chloride and using E. coli C (ATCC #13706) as host was also used to test the potable waters. 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[8].

RESULTS AND DISCUSSION

Data obtained from this study are presented in Table 1. It is obvious from these data that chlorine residuals in these distribution lines from which these drinking water samples were drawn are almost nonexistent and, when present, are at extremely low levels, 0.1 mg/L. Of the four samples showing a free chlorine residual, three contained only coliphage and the other sample was negative for both coliphage and bacteria.

Coliphage were found in 16 of the 20 samples, and nine samples, three of which had chlorine residuals, were only positive for coliphage. In only three of the samples positive for bacteria, no coliphage were detected, #1, #3 and #4. In an extensive review of the

early literature, Grabow[10] strongly suggested that most common pathogenic viruses are much more resistant to chlorination treatment than are E. coli. Chambers[11] and Havelaar[12] 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 can also survive the normal treatment and disinfection process accorded these potatble water samples[12]. Other implications of the data 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 re coliform free but coliphage containing potable waters are not single rare events. Similar results have also been reported from Singapore[6] and Cairo[7] 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 on two occasions when the TC/FC MPN combination was negative and in no instance were the TC/FC tests positive and the P/A and H_2S paper strip tests negative. However, on one occasion, the H_2S paper strip test was positive when all other bacterial tests were negative, but not the coliphage test.

These data are interesting as they indicate that the P/A and H_2S paper strip tests are equally or more sensitive for health indicator bacteria testing in potable water samples than the traditional TC MPN procedure. Similar results were found in an earlier Egyptian study[7]. Both the P/A and H_2S tests are extremely simple to carry out and in

routine and field laboratories with minimally trained staff and are also much more cost effective than traditional TC/FC MF and MPN tests. The H_2S test also appears to be equally sensitive at 22°C and 35°C.

In summary, based on these and earlier[6,7] studies 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 6 h, if necessary. It is very economical and simple to perform and its sensitivity can easily be increased in testing more 5 mL aliquots, or 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 - 1 year), they would be ideal testing procedures for isolated water supplies and where laboratory facilities do not exist.

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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 Test				TC MPN /100 mL	FC MPN /100 mL	Coliphage PFU[8] /100 mL
			TC[1]	FC[2]	FS[3]	C.p.[4]	P.a.[5]	S.a.[6]	Aero[7]	+ or neg		Bacteria Identified				
										22°	35°	22°	35°			
1	19-10	0.0	P	P	A	A	A	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	<2	<2	0
2	19-10	0.0	P	P	A	A	A	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	4	4	1
3	19-10	0.0	P	P	A	A	A	A	A	-	+		Citrobacter E. coli	12	9	0
4	19-10	0.0	P	P	A	A	P	A	A	+	+	Citrobacter Not confirmed	Citrobacter E. coli	2	2	0
5	19-10	0.0	P	P	A	A	A	A	A	-	+		Citrobacter E. coli	21	13	13
6	26-10	0.0	A	A	A	A	A	A	A	+	+	Not confirmed	Citrobacter E. coli	<2	<2	8
7	26-10	0.0	P	P	A	A	A	A	A	-	+	Citrobacter	Citrobacter E. coli	4	<2	15
8	26-10	0.0	A	A	A	A	A	A	A	-	-	Not confirmed	Citrobacter E. coli	<2	<2	9
9	26-10	0.0	P	P	A	A	A	A	A	-	-	Not confirmed	Citrobacter E. coli	2	2	5
10	26-10	0.0	A	A	A	A	A	A	A	-	-			<2	<2	2
11	2-11	0.0	A	A	A	A	A	A	A	-	-			<2	<2	1
12	2-11	0.0	A	A	A	A	A	A	A	-	-			<2	<2	12
13	2-11	0.1	A	A	A	A	A	A	A	-	-			<2	<2	3
14	2-11	0.1	A	A	A	A	A	A	A	-	-			<2	<2	57
15	2-11	0.1	A	A	A	A	A	A	A	-	-			<2	<2	1
16	9-11	0.0	A	A	A	A	A	A	A	-	-			<2	<2	4
17	9-11	0.0	A	A	A	A	A	A	A	-	-			<2	<2	14
18	9-11	0.0	P	P	A	A	A	A	A	+	+	Citrobacter E. coli	Citrobacter E. coli	21	21	9
19	9-11	0.0	P	P	A	A	A	A	A	+	+	Citrobacter E. coli	Citrobacter E. coli	26	6	2
20	9-11	0.1	A	A	A	A	A	A	A	-	-			<2	<2	0

TC[1] - total coliforms
FC[2] - fecal coliforms
FS[2] - fecal streptococci
C.p.[4] - Clostridium perfringens
P.a.[5] - Pseudomonas aeruginosa
S.a.[6] - Staphylococcus aureus
Aero[7] - Aeromonas spp
PFU[8] - plaque forming units