BACTERIOLOGICAL ANALYSIS OF DRINKING WATER

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Zumra Sami (Public Health Division, National Institute of Health, Islamabad.)
Mubashir A. Khan, Abdul Ghafoor (PMRC Central Research Centre, National Institute of Health, Islamabad.)

Abstract

A total of 112 water samples in and around Islamabad were assessed for bacteriological contamination. Forty six (81%) untreated and 21 (38%) treated water samples were positive for coliforms. Faecal coliforms and faecal streptococci were found in 26 and 6 samples, respectively, indicating contaminated sources, inadequate treatment or post-treatment contamination of drinking water. Escherichia coli was found in 26.5% of the samples which is an indicator of faecal pollution (JPMA 38: 92 , 1988).

INTRODUCTION

The most common and widespread danger associated with drinking water is contamination, either directly or indirectly, by sewage, other wastes or human and animal excrement[1]. About 25 years ago, authoritative estimates indicated that each year some 500 million people are affected by water-borne or water associated disease, and as many as 10 mfflion of these die[2]. In a recent estimate based on WHO reports suggests that 80% of all human illnesses in the dcveloping world are caused by biological contamination[3]. Faecal pollution of drinking water may introduce a variety of intestinal pathogens. Their presence being related to microbial diseases and carriers present in the community, which may cause diseases from mild gastroentritis to severe and sometimes fatal dysentry, cholera or typhoid. Other organisms, naturally present in the environment and not regarded as pathogens, may also cause opportunist disease[1]. Ideally, drinking water should not contain any microorganisms known to be pathogenic. It should be free from bacteria indicative of pollution with excreta[4]. The majority of the population in developing countries is not adequately supplied with potable water, and thus obliged to use unsafe water for domestic and drinking purposes[5] Pakistan, a developing country, is also facing a problem of wholesome water supply. This study was initiated to determine bacterial loads and contaminants in drinking water in and around Islamabad as water quality guidelines form a basis for judgement of the acceptability of public drinking water supplies[5].

MATERIAL AND METHODS

Sample collection:

One hundred and twelve water samples were aseptically collected in sterilized bottles from various sources in and around Islamabad. Sampling was done from May to October, 1984. Samples were collected from wells, springs, rivers and municipal tap water supplies. All samples were immediately transported to the laboratory and processed within two hours.

Sample Processing:

A. pH of water: pH of all the water samples was recorded by means of a pH meter.

B. Bacteriological Analysis:

i) Presumptive test for coliforms

a. Untreated water samples: Water samples were processed as described by Rand et al[6]. Briefly, five tubes of double strength lactose broth (containing durham tube) were inoculated with 10 ml water sample (in each tube) and two tubes of single strength with 1.0 ml and 0.1 ml respectively. After

incubation at 35°C for 48 hours production of acid and the presence of gas in any of the durham tubes was considered positive. Number of the positive tubes was recorded and most probable number (MPN) was calculated according to MPN tables[6].

b. Treated water samples: In case of chlorinated or sand filtered water, 50 ml of double strength Mac-Conkey's broth was inoculated with 25 ml ofwáter sample and incubated at 35°C for 48 hours[7]. The rest of the procedure was same as for untreated water samples.

ii) Confirmatory test for faecal coil-forms:

One ml from each positive tube of presumptive coliforms was inoculated in Brilliant Green Lactose bile Broth (BGLB) tube. After incubation at 44.5°C in a water bath for 24 hours; tubes with gas and turbidity were considered positive. Positive tubes were further cultured on Eosine Methylene Blue agar (EMB) for isolation of faecal coliforms[7]. Isolated colonies were confirmed by using biochemical tests[10] as well as Systek kit No.1.

iii) Confirmatory test for faecal streptococci:

Positive tubes of presumptive coliform test were subcultured in glucose broth and incubated for 2 hours at 37°C. Sodium azide (0.25 gm/500 ml) was then added and incubation carried out at 44.5°C for a further 48 hours. Positive tubes showing acid were subcultured on MacConkey agar plates and incubated at 37°C for 24 hours; The presence of small red pinpoint colonies were indicative of Streptococcus faecalls. Gram staining and the production of acid in mannitol and lactose only, but not in raffinose, confirmed their presence[8].

iv) Standard Plate Count (SPC):

The standard plate count was done by pour plate technique using 10 fold dilutions (upto 10-6) in ringers solution. One ml of each dilution was poured (duplicates) in empty, sterilized petridishes. About 12 to 15 ml of plate count agar (kept at 45°C in a waterbath) was added to each plate. Plates after solidification were incubated at 37°C for 24 to 48 hours. Plates showing 3 0—300 colonies were counted to determine the SPC per ml of sample tested. [9]

v) Analysis of other enteric pathogens.

Salmonella, Shigella and vibrio spp. Fifty ml of selenite broth and same quantity of alkaline peptone water in flasks were inoculated with 25ml water (in each flask). After incubation for 16-18 hours at 37°C subcultures from the former was made on Xylose Lysine Dextrose agar (XLD) plate and on Thiosulphate Citrate Bile salt Sucrose agar (TCBS) from the later. Plates were incubated at 37°C for 24 hours. Suspected colonies were identified biochemically and serologically[10,11].

RESULTS

The water samples investigated in this study were mainly analysed for the bacteriological contamination. The pH of the water samples (112) fall between pH 7—8.3. Water samples were analysed categorized in two different groups (Table I).

Table 1. Presumptive Test for Coliforms.

n = 112

Type of Water sample	Positive	Negative	Total
Untreated	46 (81%)	11 (19%)	57
Treated	21 (38%)	34 (62%)	55
Total	67 (60%)	45 (40%)	112

Out of 57 untreated water samples, 46 (8 1%) were found positive for the presence of coliforms while out of 55 treated water samples, 21 (38.0%) were found positive for the same. The samples which were found positive for coliform presumptive test underwent confirmatory test. Out of 67 positive samples 26 were confirmed for the presence of faecal coliforms. In addition 6 water samples were found positive for Streptococcus faecalis. Load of Viable aerobic bacteria per ml of the water sample was determined, (Table II).

Table III. Bacterial Isolates recovered from positive Samples.

Isolates	Treated water samples	Untreated water samples	Total isolates
	No.	No.	No.
E. Coli	10 (32%)	16 (24.5%)	26 (27%)
Streptococcus faecalis	2 (6.5%)	4 (6 %)	6 (6%)
Staphylococcus aureus	1 (3 %)	1 (1.5%)	2 (2%)
Pseudomonas spp.	7 (22.5%)	12 (18.5%)	19 (20%)
Other enteric bacteria	2 (6.5%)	12 (18.5%)	14 (14.5%)
Other environmental bacteria	9 (29 %)	20 (31 %)	29 (30 %)
Total	31 (32 %)	65 (68%)	96 .

Twenty nine (26%) specimens showed no growth on the plate count agar and were mostly from treated water samples. Other samples enumerated counts between < 10 to> 106/ml. Escherichia coli (27%) was the major pathogen among the other bacterial isolates (Table III)

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followed by Streptococcus faecalis (6%) and Staphylococcus aureus (2%). Other organisms were Pseudomonas Spp. (20%), enteric bacteria (14.5%) and environmental bacteria (30%). Some of the samples contained more than one isolate. All the water samples investigated for the presence of Salmonella, Shigella and Vibrio cholerae were found negative.

DISCUSSION

An acceptable pH for drinking water is between pH 6.5 to pH 8.5, recommended by WHO as a guideline value and in the absence of a distribution system acceptable range may. be broader. AU the water samples examined in this study were in acceptable pH range.

For the presumptive coliforms test, the WHO guideline for both treated and untreated water samples is 0/ 100 ml [4], but in an occasional untreated water sample 3 coliform/100 ml are allowed on the condition that these would not be found in consecutive water samples. [12] The coliform group as an indicator bacteria are used to evaluate the potability of drinking water and the presence of any coliform organisms is an indication of a contaminated source, inadequate treatment or post treatment contamination[13]. In unpiped water supplies, sometimes upto 10 coliforms/l00 ml are allowed but they should not occur repeatedly; if occurrence is frequent and sanitary protection cannot be improved, an alternative source must be found if possible. [12] In this study 81 % of the untreated and 38% of the treated water samples were positive for MPN, showing a high contamination and risk to public health The detection of faecal (Thermotolerant) coliform organisms provide definite evidence of faecal pollution[4] and they were found in 39% of the positive samples. Search for Streptococcus faecalis is not carried out routinely. Its main value is when doubt is expressed that large number of irregular types of coliforms found in a sample of water are of faecal origin. Confirmation of faecal pollution would then rely on finding £ faecaiis in the water[8]. Since they survive longer in water than coliform bacteria they should be referred as indicator of faecal pollution in water and shelfish.[14] In the present study they were found only in 6% of the water samples examined. The main value of colony counts lies in the comparison of results obtained from regular samples from the same supply so that any significant change from the normal range in a particular location can be detected.[1] As the SPC in most of the untreated water samples (Table II) was very high, it is therefore desirable to disinfect all supplies of drinking water before distribution. Supplies derived from protected sources which are distributed without disinfection should be similar in quality to that of disinfected drinking water. Where it is impracticable to supply water to consumers through a piped distribution network and where untreated sources such as wells, bore-holes and springs which may be naturally pure must be used, considerable reliance should be placed on sanitary examination and not exclusively on the results of bacteriological examination.[15] The high percentage of E. coil (27%) provides a definite evidence of faecal pollution in water.[4] Staphylococcus aureus (2%) which is relatively recently accepted as indicator organisms in food and water, provides a useful indication that faecal contamination has occurred in water.[14] Pseudomonas spp. are common inhabitant of soil and water and found in small numbers in the faeces of man and animals. These were isolated in about 20% samples and are of public health importance as some' species cause a variety of suppurative infections in man. Enterotoxigenic strains of pseudomonas spp. alongwith other species of Enterobacter, Kiebsiella and Acinetobacter have been isolated from cases of infantile diirrhoea in Addis Ababa during surveys in 1974 and 1977.[16,17,18] Enterotoxigenic species of proteus with other enterotoxigenic bacteria have also been reported in a study on food and water from an Ethiopian community.[19] Environmental bacteria (Table Ill) include Alcaligenes spp., Acinetobacter spp., Yeast spp. and Bacillus spp. which are usually found in soil. Presence of such high bacterial counts and presence of faecal coliforms and other indicator organisms as Streptococcus faecaiis, Staphy - lococcus aureus indicate inadequate treatment, post treatment contamination and contaminated water sources. Therefore, everything possible should be done to prevent pollution of the drinking water, special attention being given to the safe disposal of excrement. But the significance of routes of transmission other than drinking water should not be underestimated as the provision of a safe potable water supply by itself will not necessarily prevent infection without accompanying improvement in

sanitation and personal habits. Education in simple hygiene is also essential.

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