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QUALITATIVE ANALYSIS OF DRINKING WATER OF LAHORE FOR THE PRESENCE OF COLIFORM BACTERIA

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Abstract: Water samples, from direct supplies and overhead tanks of different localities in Lahore were collected and analyzed for the presence of coliform bacteria by using lactose broth and Endo agar media. It was observed that almost all of the samples harboured coliform bacteria indicating some forms of fecal pollution. The results show that either the water-storage or water pipe lines are not being managed properly.

Key words: Drinking water, pollution, coliforms, lactose fermenting bacteria, *E. coli*.

INTRODUCTION

The quality of water, for both drinking and recreation purposes is now a matter of national and international concern (Collins *et al*., 1995). Every impact which changes the quality of our surface and subsoil waters to such a degree that turns it unsuitable either for human consumption or for survival of fauna and flora is considered water pollution (Chow, 1964; Ramalho, 1977; Eckenfelder, 1989; Chhatwal *et al*., 1989; Shukla and Shrivastiva, 1992; Hussain, 1998). Odours from polluted waters and trash in the water system affect quality of life, tourism and economic development. Water pollution harms aquatic life and ecological balance. During and after rain, runoff carries pollutants including fecal coliform bacteria, pesticides, fertilizers, chemicals, oil and grease etc., into water ways. Contamination also occurs from sanitary sewer systems and waste water treatment plants (Edmonds, 1978; Dufour, 1984; Sterrit and Lester, 1988).

Water that contains large numbers of bacteria may be perfectly safe to drink. The important consideration, from a microbiological standpoint, is the kinds of microorganisms that are present. Water from streams and lakes, which contains multitudes of autotrophs and saprophytic heterotrops is potable as long as pathogens for human are lacking. The intestinal pathogens such as those that cause typhoid fever, cholera and bacillary dysentery are of prime concern. The fact that human fecal material is carried away by water in sewage systems that often empty into rivers and lakes presents a colossal sanitary problem, making constant testing of municipal water supplies for the presence of fecal microorganisms as essential procedure for the maintenance of water purity (Benson, 1994). Bacterial contamination cannot be detected by sight, smell or taste. Therefore, a water supply has to be tested through microbiological methods. Coliform bacteria have been used to assess the quality of water and presence of pathogens. Although several of

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the coliform bacteria are not usually pathogenic themselves but they serve as an indicator of potential bacterial pathogens contamination. The simpler, quicker and safer nature of the method for analyzing these microorganisms as compared to the efforts required to verify the presence of individual pathogens, has made it a popular routine water assay protocol (Gaudy and Gaudy, 1980; USEPA, 1986; Francy *et al.*, 1993).

There are a number of methods and media available for the detection and enumeration of indicator microorganisms, such as multiple-tube most probable number fermentation technique (MPN), membrane filter method, presence/absence test and employing defined substrate (AWWA, APHA and WEF, 1992; Benson, 1994; Collins *et al.*, 1995). The present study was intended to process drinking water from direct supplies and over-head tanks of different areas of Lahore, for the presence of coliform bacteria. The informations are relevant to public-health authorities.

MATERIALS AND METHODS

Samples of drinking water were collected from different areas of Lahore (Table 1). Direct supply lines and as well as over-head tank of houses, were sampled by taking about 100 ml of water in sterile glass bottles.

Qualitative analysis of the samples for coliform bacteria

Water samples were analyzed by multiple-tube most probable number (MPN) fermentation technique (Benson, 1994; Collins *et al.*, 1995). The procedure was completed in three steps named, presumptive, confirmed and completed tests.

Presumptive test

Double strength and single strength lactose broth were prepared according to Merck (1996/97). Single strength lactose broth (SSLB) contained (% W/V) 0.5 each of peptone and lactose, and 0.3 meat extract. The double strength lactose broth (DSLB) contained double amounts of all the ingredients. For a given sample nine test tubes were fitted with Durham's tubes and divided into three sets. 10 ml of DSLB was dispensed into each of the three test tubes of one set, and SSLB into other two sets. The tubes were cotton plugged and sterilized by autoclaving. On cooling down the DSLB-test tubes were inoculated with 10 ml, one set of SSLB with 1 ml and the other with 0.1 ml of a water sample. The inoculated tubes were incubated at 37°C for 24-48 hours, and then observed for gas production. The most probable number (MPN) of coliform bacteria was determined from MPN table (Benson, 1994).

Confirmed test

Endo agar medium was prepared by mixing 3.6 gm of Endo agar (Oxide) and 0.4 ml of 10% (W/V) alcoholic basic fuchsin in 100 ml of distilled water. The medium was autoclaved and allowed to cool down to about 50°C before pouring in pre-sterile petri plates. Following solidification the petri plates were streaked on from the cultures of lactose broth. The plates were incubated for 24 hours at 37°C and observed for the presence of coliforms' colonies.

Completed test

Bacterial colonies from the Endo agar plates were subcultured into lactose broth fermentation tubes and on nutrient agar slopes. Both were incubated at 37°C for 24 hours. Gas production was noted in the test tubes, while growth on nutrient agar slants was processed for Gram's staining.

Table I: Drinking water sampling sites and dates.

Sample No.	Sampling locality	Collection date
1.	Mian Meer Colony	15.09.2000
2.	Gulshan-e-Ravi	21.09.2000
3.	Zoology Department, Punjab University, Lahore	21.09.2000
4.	Thokar Niaz Baig	11.10.2000
5.	Ghari Shahoo	11.10.2000
6.	Faisal Town	12.10.2000
7.	Allama Iqbal Town	14.10.2000
8.	Rehman Pura	16.10.2000
9.	Dharam Pura	20.10.2000
10.	Jain Mandar	20.10.2000
11.	Davis Road	26.10.2000
12.	Sanda	28.10.2000
13.	Ichhra	01.11.2000
14.	Karim Park	03.11.2000
15.	Samanabad	04.11.2000
16.	Sabzazar	20.11.2000
17.	Shadbagh	21.11.2000

RESULTS

Presumptive test

Sparing the three samples for which stored water was not available about 36% of the samples showed gas production both in the direct supplies as well as in the stored water. About 29% of the samples yielded gas in stored water only. 7% of the samples indicated gas production only in direct supplies. 29% samples did not give the positive reaction at all (Fig.1).

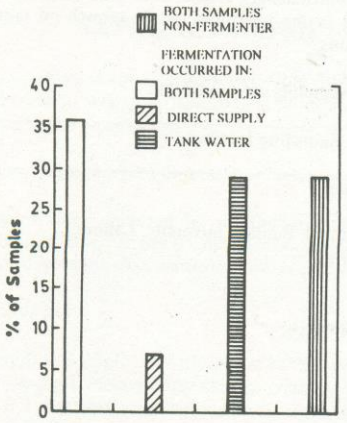

Fig. 1: Incidence of lactose fermentation by water samples.

Results of the presumptive test showed that water of both direct supplies as well as over-head tanks of sample number 8, 10, 11, 12 and 14 produced gas in lactose broth. Samples number 6, 9, 15 and 16 were found negative for the gas production at all. Four samples *i.e.*, 1, 2, 3 and 7 indicated gas production only in overhead tank water. For sample number 13, the direct supply gave positive reaction, while the overhead tank water did not produce gas. In case of samples, 4, 5 and 17 only direct supplies were sampled and they all showed positive results. Most probable number of lactose fermenter bacteria of these samples, are shown in Table II and Fig.2

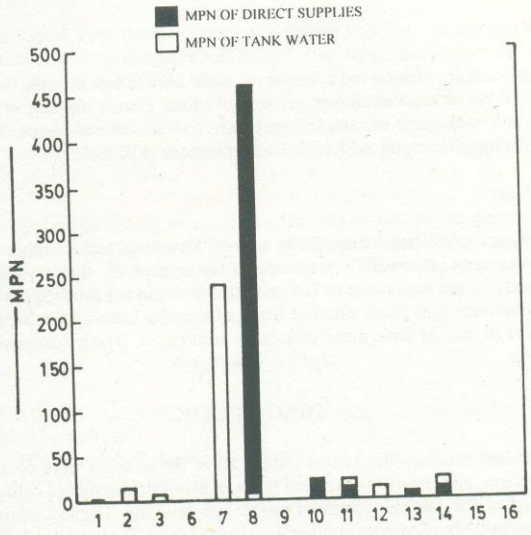

Fig. 2: Graph showing most probable number (MPN) of water samples.

Table II: Indication of coliform bacteria in water samples of direct supplies from pipelines (D.S.) and household overhead tanks (T.W.) of different localities of Lahore.

Sample	[a]MPN/ 100 ml of sample		Confirmed Test on Endo agar				Completed Test			
			Pink/red colonies		Metallic sheen of colonies		Gas production		Grams' staining	
	D.S.	T.W.	D.S.	T.W.	D.S.	T.W.	D.S.	T.W.	D.S.	T.W
1[c]	-	4	+	+	-	-	-	-	g-ve rods	b-ve
2	-	15	+	+	-	-	-	+	g-ve rods	b-ve
3	-	7	+	+	-	-	-	+	g-ve rods	b-ve
4	1100+	•	+	•	+	•	+	•	g-ve rods	•
5	4	•	+	•	-	•	-	•	g-ve rods	•
6	-	-	+	+	-	-	-	-	g-ve rods	b-ve
7	-	240	+	+	-	+	-	+	g-ve rods	b-ve
8	460	9	+	+	-	-	+	-	g-ve rods	b-ve
9	-	-	+	+	-	-	-	-	g-ve rods	b-ve
10	23	4	+	+	-	-	+	-	g-ve rods	b-ve
11	21	23	+	+	+	+	+	+	g-ve rods	b-ve
12	15	15	+	+	-	-	+	+	g-ve rods	b-ve
13	9	-	+	+	-	-	-	-	g-ve rods	b-ve
14	15	23	+	+	-	-	+	+	g-ve rods	b-ve
15	-	-	+	+	-	-	-	-	g-ve rods	b-ve
16	-	-	-	-	-	-	-	-	g-ve rods	b+ve cocci
17	240	•	+	•	+	•	+	•	g-ve rods	•

[a]: most probable number of coliforms; [b]: amount (ml) of sample; b-: gram-ve bacilli; c+: gram+ve cocci; •: sample was not available; -: negative for fermentation; +: positive for fermentation.

Confirmed test

All the samples yielded red colonies on Endo agar except sample 16, irrespective to their status of gas production during presumptive tests. Direct supplies of sample number 4 and 17, while tank water of sample 7 and both types of water of sample 11, gave rise red colonies with metallic sheen, which indicated presence of *E. coli*.

Completed test

Inoculation of colonies from Endo agar to lactose fermenter tubes showed results similar to the ones obtained in presumptive test except for direct supplies of sample number 5 and 13, and tank water of 1, 8 and 10, which did not produce gas at this step. All cultures on nutrient agar slant, streaked from colonies on Endo agar represented Gram-ve bacilli except in case of tank water of sample number 16, which comprised of Gram+ve staphylococci.

DISCUSSION

The present results indicate that almost all of the samples were contaminated with coliform bacteria, and regarding their MPN (most probable number of coliforms/100 ml of sample) most of the samples appeared unsafe for drinking. Highest contamination was noted in direct supply of sample number 4, collected from Thokar Niaz Baig as envisaged by a figure of more than one thousand for MPN. All the colonies of this sample on Endo agar possessed metallic sheen, which indicated the presence of *E. coli* in the water. Similarly, direct supplies of sample numbers 8 and 17 and overhead tank water of 7 were also highly contaminated with MPN values of 460, 240 and 240, respectively. Colonies of sample numbers 7, 11 and 17 also expressed metallic sheen. Remaining samples indicated 4 to 23 MPN (Table II, Fig.2). These all values are higher than the established safe level for drinking water, which is 3 MPN (Baker and Breach, 1980).

It was noticed that all the samples except No. 16 irrespective to their lactose fermenter/ non-fermenter nature formed pink to red colonies on Endo agar. Although the Endo agar medium has been considered selective for lactose fermenters (Benson, 1994), but it is also reported that the medium is slightly selective one and some enteric pathogens such as *Salmonella* may form faint pink colonies, while *Shigella* may develop slightly pinker colonies (Rohde, 1973). *Shigella* as well as *Salmonella* are non-fermenters of lactose and Gram-negative bacilli (Benson, 1994; Collins *et al.*, 1995). Emergence of pink colonies on Endo agar for the samples No. 6, 9 and 15, which appeared non-lactose fermenter during the presumptive as well as completed tests indicate the presence of such enteric pathogens. Similarly, for sample numbers 1-3 and 7, the water taken from the direct supplies did not produce gas during the presumptive and the completed tests but pink to red bacterial colonies appeared on the Endo agar medium. A similar situation was observed for tank water of the sample No.13. These results strongly suggest that while

analyzing water samples for the prevalence of coliform bacteria, the sample, which yield no gas production at the presumptive test stage, must be processed further on selective media such as Endo agar and EMB etc., to monitor the presence of enteric non-fermenter pathogens. It is important to note at this level that many workers in their protocols for the analyses of water samples for coliforms have mentioned that if no gas is produced by a sample at presumptive test stage, the samples need no further processing (Benson, 1994; Collins *et al*., 1995; Black, 1996).

From the results obtained it is recommended that almost all the water samples are unsafe for drinking purpose and many human gastrointesfinal diseases might have been prevailing in these areas. These information, however, suffice to open the eyes of public health authorities to feel their responsibilities in relation to the provision of safe drinking water for the urban population.

REFERENCES

BAKER, F.J. AND BREACH, M.R., 1980. *Medical Microbiology*. Butterworth & Co. (Publishers) Ltd., London, pp.434-436.

BENSON, H.J., 1994. *Microbial Applications, Laboratory Manual in General Microbiology*. W.M.C. Brown Publishers, Dubuque, U.S.A., pp.206-209.

BLACK, J.G., 1996. *Microbiology, Principles and Applications*. Prentice Hall, Inc., New Jersey, U.S.A., pp.726-754.

CHHATWAL, G.R., MEHRA, M.C., SATAKE, M., KATYAL, T., KATYAL, M. AND NAGAHIRE, T., 1989. *Encyclopedia of Environmental Pollution and its Control*, Vol.1-6. Anmol Publishers, Ansari Road, New Delhi-110002, India, pp.60.

CHOW, V.T., 1964. *Handbook of Applied Hydrology*. McGraw-Hill Book Company, New York, U.S.A.

COLLINS, C.H., LYNE, P.M. AND GRANGE, J.M., 1995. *Microbiological Methods*. Butterworth-Heinemann Ltd., London, pp.270-280.

DUFOUR, A.P., 1984. *Health Effects Criteria for Fresh Recreational Waters*. USEPA, Cincinnati, OH, EPA 600/1-84-004.

ECKENFELDER, W.W., 1989. *Industrial Water Pollution Control*. McGraw-Hill Book Company, Singapore.

EDMONDS, P., 1978. *Microbiology. An Environmental Perspective*. MacMillan Publishing Co.

FRANCY, D.S., MYERS, D.N. AND METZKER, K.D., 1993. *Escherichia coli and Fecal-Coliform Bacteria as Indicators of Recreational Water Quality*. U.S. Geological Survey.

GAUDY, A.F. AND GAUDY, E.T., 1980. *Microbiology for Environmental Scientists and Engineers*. McGraw-Hill Book Co.

HUSSAIN, M., 1998. *Environmental Degradation: Realities and Remedies*. Ferozesons (Pvt.) Limited, 60-Shahrah-e-Quaid-e-Azam, Lahore, Pakistan, pp.10.

MERCK, 1996/97. *Microbiology Manual*, pp.70.

RAMALHO, R.S. (ed.), 1977. *Introduction to Waste Water Treatment Process*. Academic Press, New York, USA.

ROHDE, P.A. (ed.), 1973. *BBL Manual of Products and Laboratory Procedures*. Cockeysville, Maryland 21030, U.S.A., pp.107.

SHUKLA, S.K. AND SRIVASTIVA, P.R., 1992. *Water Resources, Planning and Development*. Commonwealth Publishers, New Delhi, India.
STERRIT, P.M. AND LESTER, J.N., 1988. *Microbiology for Environment and Public Health Engineers*. E. & F.N. Spon, London, pp.234-254.
U.S. ENVIRONMENTAL PROTECTION AGENCY (USEPA), 1986. *Ambient Water Quality Criteria for Bacteria* 1986. EPA 440/5-84-002.

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