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RESEARCH ARTICLE

Bacteriological Quality Analysis of Tap Water of Karachi, Pakistan

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Abstract

Tap water supplies in Pakistan comes from lakes, well, and springs. Tap water samples were studied to assess their bacteriological characteristics and suitability for potable purposes. A cross-sectional epidemiological method was adopted to investigate the tap water of ten main localities of Karachi. The bacteriological examination of water samples included the most probable number of presumptive coliforms, faecal coliforms, and faecal streptococci. The results showed that the total coliform count was detected in all samples of water taken from Karachi Pakistan. Another methods Escherichia coli (EC) medium and Membrane Filtrations Technique also used for the detection of coliforms. In both methods coliforms presence were indicated except sample number eight, that was purified bottle water. The most common group of indicator organisms used in water quality monitoring are coliforms. These organisms are representative of bacteria normally present in the intestinal tract of mammals including human. Contamination of water may occur during its transportation from the plant to the consumer or during storage in a house reservoir. Improving and expanding the existing water treatment and sanitation systems is more likely to provide good, safe, and sustainable sources of water in the long term.

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INTRODUCTION

Pure and clean water is one of the several basic necessities of civilized human population and it is an undeniable fact that germ free safe water brings high standards to the public health. In Pakistan most drinking water supplies come from rivers, lakes, well, and springs and the availability of safe water is only 40 to 60%. Such natural water supplies, particularly streams and rivers, are likely to be polluted with domestic and industrial wastes, as the used water of a community is ultimately disposed off in these natural drains. It is not the mere number of microbes that affects water quality, as water containing large number of harmless bacteria may yet be safe for drinking, but it is the kind of specific microorganisms that is determinative (Benson, 1994; Farnleitner et al., 2001; Said et al., 2004). Because water borne diseases are caused by pathogenic microorganisms, therefore it becomes very important to find out the bacteriological condition of drinking water to ensure its safety. Potable water is subjected to a series of processes of water treatment including sedimentation, coagulation, filtration and chlorination. Before the consumption of water, it seems pertinent that the perfect safety and satisfactory consumption of the material should be ensured. This requires the application of a methodology to establish the water safety and to evaluate the efficiency and effectiveness of treatment procedures. The safety can be established on the basis of detection of coliforms. It has been established

that routine examination of water for the presence of intestinal pathogens would be a tedious and difficult task, while it is much easier to demonstrate the presence of some non pathogenic intestinal types such as *Escherichia coli* or *Streptococcus faecalis*, since these organisms are always found in the intestine and are normally not present in soil or in water.

Thus it can be assumed that their presence in water indicates that the fecal material has contaminated the water supply. These bacteria also survive a little longer than the enteric pathogens (Pelczar et al., 1986; Benson, 1994; Muneer et al., 2001;Tantawiwat et al., 2005). The coliforms are Gram-negative, motile, or nonmotile, nonsporing, rod-shaped bacteria. They are aerobic or facultative anaerobic that ferment lactose with gas formation within 48 hours at 35 °C. They are commonly found in intestinal tract of men and animals. The coliforms are considered as indicator organisms and are used all over the world to establish the degree of fecal pollution in water. The coliform bacteria include the members of the genera *Escherichia*, *Enterobacter*, *Proteus*, *Yersinia*, *Hafnia*, *Serratia* and *Klebsiella*. Most important of all are *E. coli* and *Enterobacter aerogenes* which are abundantly found as commensals in intestinal tract and thus are regularly discharged in the feces by all humans. Any material which is focally polluted will definitely contain *E. coli* and *Enterobacter aerogenes*. In other words, the presence of these coliforms in any material indicates that the material is fecally polluted (Levine et al., 1918).

Presence of coliforms is determined by performing the coliform tests which include several methods. Most probable number (MPN) test is a specific test to determine the presence of coliforms in a given sample. The coliforms are detected on the basis of their characteristic capability of fermenting lactose with the production of gas. The enteric bacteria other than coliforms do not ferment lactose, if ferment, they do not produce gas. *E. coli* (EC) medium (Otaibi., 2009) is used for testing water, milk, shellfish, and other materials for evidence of fecal contamination. EC medium has shown to be excellent for the isolation of coliform bacteria at 37°C and of *E. coli* at 45.5°C and later with the modification for rapid screening of *E. coli* detection (Goel., 1997). The membrane filter (MF) technique is highly reproducible, can be used to test relatively large volumes of sample, and yields numerical results more rapidly than the multiple-tube procedure. The MF technique is extremely useful in monitoring drinking water and a variety of natural water. The MF Technique is also used for microbial monitoring in the pharmaceutical, cosmetics, electronics, and food and beverage industries. It also allows for removal of bacteriostatic or cidal agents that would not be removed in Pour Plate, Spread Plate, or MPN techniques. As related to the membrane filter technique, the coliform group may be defined as comprising all aerobic and many facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that develop a red colony with a metallic sheen within 24h at 35°C on an Endo-type medium containing lactose. Some members of the total coliform group may produce a dark red or nucleated colony without a metallic sheen (Taneja et al).

Materials and methods

Samples Collection

Samples of tap water were collected from ten different localities of Karachi, Pakistan (Table 1). All Samples were collected in sterile sample collection bottles and labeled with respective sample number.

Table 1: Localities of samples collected in the study:

| S. No. | Sample ID | Locality |
|--------|-----------|-----------------------|
| 1 | 01 | North Nazimabad |
| 2 | 02 | Orangi Town |
| 3 | 03 | Landhi |
| 4 | 04 | Fedral B Area |
| 5 | 05 | Martin Road |
| 6 | 06 | MalirCantt |
| 7 | 07 | Lyari |
| 8 | 08 | Purified water bottle |
| 9 | 09 | Karachi University |
| 10 | 10 | Gizri Clifton |

Bacteriological Analysis

Detection of coliforms by Most Probable Number (MPN) Test

Measured aliquots of the samples to be tested were transferred to a series of three groups (one double strength and two single strength) of lactose and nutrient broths 10ml, 1ml, and 0.1ml, respectively. Each group lactose and nutrient broths composed of three tubes of specified medium.

Detection of coliforms by Escherichia coli (EC) medium

EC broth was used for *E. coli* detection in water samples. 0.1 ml of the liquid from the positive presumptive tube was transferred to the fermentation tube containing EC broth medium. Within 30 minutes of inoculation, inoculated EC broth tube(s) were incubated in a water bath at 44.5°C for 24 hours. Development of turbidity in the fermentation tubes and presence of gas in the Durham tubes were considered positive evidence of fecal coliforms in water samples.

Detection of coliforms by Membrane Filtration Technique

MF technique was used to test large volumes of sample. The membrane filter method was made possible by the invention of the membrane filter, a thin filter which is capable of holding microorganisms while allowing water to pass through. Forceps was sterilized in alcohol, air dried, then used to remove the membrane filter from the package. The filter was then centered on the holder base, grid side up. The filter funnel was placed onto the assembly and secured. 100 ml of sample water was poured or pipette into the funnel of the filtration unit. Then water was vacuumed from the surface of the filter. Forceps were used to remove the membrane from the holder and placed it onto the media surface, ensuring that there were no air bubbles between the filter and the media. Eosin Methylene Blue (EMB) medium was used. The Petri dishes containing the filter were placed in an incubator at 35°C for 24 hours.

Results

Analysis of collected tap water samples by three different methods, MPN, EC medium, and MF Technique indicated that samples were fecally contaminated.

The MPN test provided presumptive evidence of the presence of coliforms. The coliforms were detected on the basis of their characteristic capability of coliforms. The MPN was estimated by determining the number of tubes in each group that showed gas formation following the appropriate incubation (Table 2)

Table: 2 MPN DETERMINATION FROM MULTIPLE TUBES TEST

| NUMBER OF TUBES GIVEN POSITIVE REACTION OUT OF | | | MPN INDEX PER 100ml | 95 PERCENT CONFIDENCE LIMITS | |
|--|-------------------|---------------------|---------------------------|------------------------------|-------|
| 3 of 15 ml each | 2 of 1 ml each | 3 of 0.1 ml each | | LOWER | UPPER |
| 000 | 000 | 000 | 0 | 0 | 0 |
| 001 | 000 | 000 | 1 | 0 | 1 |
| 002 | 000 | 000 | 2 | 0 | 2 |
| 003 | 000 | 000 | 3 | 0 | 3 |
| 004 | 000 | 000 | 4 | 0 | 4 |
| 005 | 000 | 000 | 5 | 0 | 5 |
| 006 | 000 | 000 | 6 | 0 | 6 |
| 007 | 000 | 000 | 7 | 0 | 7 |
| 008 | 000 | 000 | 8 | 0 | 8 |
| 009 | 000 | 000 | 9 | 0 | 9 |
| 010 | 000 | 000 | 10 | 0 | 10 |
| 011 | 000 | 000 | 11 | 0 | 11 |
| 012 | 000 | 000 | 12 | 0 | 12 |
| 013 | 000 | 000 | 13 | 0 | 13 |
| 014 | 000 | 000 | 14 | 0 | 14 |
| 015 | 000 | 000 | 15 | 0 | 15 |
| 016 | 001 | 000 | 16 | 1 | 16 |
| 017 | 002 | 000 | 17 | 1 | 17 |
| 018 | 003 | 000 | 18 | 1 | 18 |
| 019 | 004 | 000 | 19 | 1 | 19 |
| 020 | 005 | 000 | 20 | 1 | 20 |
| 021 | 006 | 000 | 21 | 1 | 21 |
| 022 | 007 | 000 | 22 | 1 | 22 |
| 023 | 008 | 000 | 23 | 1 | 23 |
| 024 | 009 | 000 | 24 | 1 | 24 |
| 025 | 010 | 000 | 25 | 1 | 25 |
| 026 | 011 | 000 | 26 | 1 | 26 |
| 027 | 012 | 000 | 27 | 1 | 27 |
| 028 | 013 | 000 | 28 | 1 | 28 |
| 029 | 014 | 000 | 29 | 1 | 29 |
| 030 | 015 | 000 | 30 | 1 | 30 |
| 031 | 016 | 001 | 31 | 1 | 31 |
| 032 | 017 | 002 | 32 | 1 | 32 |
| 033 | 018 | 003 | 33 | 1 | 33 |
| 034 | 019 | 004 | 34 | 1 | 34 |
| 035 | 020 | 005 | 35 | 1 | 35 |
| 036 | 021 | 006 | 36 | 1 | 36 |
| 037 | 022 | 007 | 37 | 1 | 37 |
| 038 | 023 | 008 | 38 | 1 | 38 |
| 039 | 024 | 009 | 39 | 1 | 39 |
| 040 | 025 | 010 | 40 | 1 | 40 |
| 041 | 026 | 011 | 41 | 1 | 41 |
| 042 | 027 | 012 | 42 | 1 | 42 |
| 043 | 028 | 013 | 43 | 1 | 43 |
| 044 | 029 | 014 | 44 | 1 | 44 |
| 045 | 030 | 015 | 45 | 1 | 45 |
| 046 | 031 | 016 | 46 | 1 | 46 |
| 047 | 032 | 017 | 47 | 1 | 47 |
| 048 | 033 | 018 | 48 | 1 | 48 |
| 049 | 034 | 019 | 49 | 1 | 49 |
| 050 | 035 | 020 | 50 | 1 | 50 |
| 051 | 036 | 021 | 51 | 1 | 51 |
| 052 | 037 | 022 | 52 | 1 | 52 |
| 053 | 038 | 023 | 53 | 1 | 53 |
| 054 | 039 | 024 | 54 | 1 | 54 |
| 055 | 040 | 025 | 55 | 1 | 55 |
| 056 | 041 | 026 | 56 | 1 | 56 |
| 057 | 042 | 027 | 57 | 1 | 57 |
| 058 | 043 | 028 | 58 | 1 | 58 |
| 059 | 044 | 029 | 59 | 1 | 59 |
| 060 | 045 | 030 | 60 | 1 | 60 |
| 061 | 046 | 031 | 61 | 1 | 61 |
| 062 | 047 | 032 | 62 | 1 | 62 |
| 063 | 048 | 033 | 63 | 1 | 63 |
| 064 | 049 | 034 | 64 | 1 | 64 |
| 065 | 050 | 035 | 65 | 1 | 65 |
| 066 | 051 | 036 | 66 | 1 | 66 |
| 067 | 052 | 037 | 67 | 1 | 67 |
| 068 | 053 | 038 | 68 | 1 | 68 |
| 069 | 054 | 039 | 69 | 1 | 69 |
| 070 | 055 | 040 | 70 | 1 | 70 |
| 071 | 056 | 041 | 71 | 1 | 71 |
| 072 | 057 | 042 | 72 | 1 | 72 |
| 073 | 058 | 043 | 73 | 1 | 73 |
| 074 | 059 | 044 | 74 | 1 | 74 |
| 075 | 060 | 045 | 75 | 1 | 75 |
| 076 | 061 | 046 | 76 | 1 | 76 |
| 077 | 062 | 047 | 77 | 1 | 77 |
| 078 | 063 | 048 | 78 | 1 | 78 |
| 079 | 064 | 049 | 79 | 1 | 79 |
| 080 | 065 | 050 | 80 | 1 | 80 |
| 081 | 066 | 051 | 81 | 1 | 81 |
| 082 | 067 | 052 | 82 | 1 | 82 |
| 083 | 068 | 053 | 83 | 1 | 83 |
| 084 | 069 | 054 | 84 | 1 | 84 |
| 085 | 070 | 055 | 85 | 1 | 85 |
| 086 | 071 | 056 | 86 | 1 | 86 |
| 087 | 072 | 057 | 87 | 1 | 87 |
| 088 | 073 | 058 | 88 | 1 | 88 |
| 089 | 074 | 059 | 89 | 1 | 89 |
| 090 | 075 | 060 | 90 | 1 | 90 |
| 091 | 076 | 061 | 91 | 1 | 91 |
| 092 | 077 | 062 | 92 | 1 | 92 |
| 093 | 078 | 063 | 93 | 1 | 93 |
| 094 | 079 | 064 | 94 | 1 | 94 |
| 095 | 080 | 065 | 95 | 1 | 95 |
| 096 | 081 | 066 | 96 | 1 | 96 |
| 097 | 082 | 067 | 97 | 1 | 97 |
| 098 | 083 | 068 | 98 | 1 | 98 |
| 099 | 084 | 069 | 99 | 1 | 99 |
| 100 | 085 | 070 | 100 | 1 | 100 |

FROM: STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, TWELFTH EDITION. (NEW YORK: THE AMERICAN PUBLIC HEALTH ASSOCIATION, INC., p. 808)

According to above standard table of MPN Test, we determined our results which showed that water collected from different localities of Karachi was non potable except sample 08 which was purified water.

Table 3: Standard MPN chart

| Sample | Acid and gas (AG) | | | | | | | | | Reading | MPN | Range 95% probability | Potability |
|--------|-------------------|---|---|--------|---|---|----------|---|---|---------|--------|-----------------------|------------|
| | LB2X-10 | | | LB1X-1 | | | LB1X-0.1 | | | | | | |
| Tubes | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | | | |
| 01 | + | + | + | + | + | + | + | + | - | 3,3,2 | 1,100 | 150-4,800 | Nonpotable |
| 02 | + | + | + | + | + | + | + | + | + | 3,3,3 | >2,400 | - | Nonpotable |
| 03 | + | + | + | + | + | + | + | + | + | 3,3,3, | >2,400 | - | Nonpotable |
| 04 | + | + | - | + | + | - | - | - | + | 2,2,1 | 28 | 10-150 | Nonpotable |
| 05 | + | + | + | + | + | + | + | + | + | 3,3,3 | >2,400 | - | Nonpotable |
| 06 | + | + | + | + | + | + | + | + | + | 3,3,3 | >2,400 | - | Nonpotable |
| 07 | + | + | + | + | + | - | + | + | - | 3,2,2 | 210 | 35-470 | Nonpotable |
| 08 | - | - | + | - | - | - | - | - | - | 1,0,0 | 4 | <0.5-20 | Potable |
| 09 | + | + | + | + | - | - | + | - | - | 3,1,1 | 75 | 14-230 | Nonpotable |
| 10 | + | - | + | + | - | + | - | - | - | 2,2,0 | 21 | 4-47 | Nonpotable |

Table 4: MPN by using lactose broth

| Samples | Acid and gas (AG) | | | | | | | | | Reading | MPN | Range 95% probability | Potability |
|---------|-------------------|---|---|--------|---|---|----------|---|---|---------|-------|-----------------------|------------|
| | NB2X-10 | | | NB1X-1 | | | NB1X-0.1 | | | | | | |
| Tubes | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | | | | |
| 01 | + | + | + | + | + | + | + | + | - | 3,3,2 | 1,100 | 150-4,800 | Nonpotable |
| 02 | + | + | + | + | + | - | - | - | - | 3,2,0 | 93 | 15-380 | Nonpotable |
| 03 | + | + | - | + | - | + | + | - | - | 2,2,1 | 28 | 10-150 | Nonpotable |
| 04 | + | + | + | + | + | - | - | - | - | 3,2,0 | 93 | 15-380 | Nonpotable |
| 05 | + | + | + | - | + | + | - | - | + | 3,2,1 | 150 | 30-440 | Nonpotable |
| 06 | + | + | + | + | + | - | + | + | - | 3,2,2 | 210 | 35-470 | Nonpotable |
| 07 | - | + | + | + | + | + | - | - | - | 2,3,0 | - | - | Nonpotable |
| 08 | - | - | - | - | - | - | - | - | - | 0,0,0 | <1 | <0.5-9 | Potable |
| 09 | - | - | - | + | + | + | + | + | + | 0,3,3 | - | - | Nonpotable |
| 10 | + | + | + | - | + | + | + | - | - | 3,2,1 | 150 | 20-440 | Nonpotable |

The above MPN results showed the high number of coliforms present in tap water. In lactose broth all samples of water showed MPN index above the limit except sample number 08 which showed very less MPN index (four) of coliforms. Sample 08 is a purified bottle branded water According to WHO drinking water standard limit for MPN test is less than 1.1 MPN/ml therefore it is also not a standard drinking water. Nutrient broth also showed high MPN index which is not according to standard. Development of turbidity in the fermentation tubes and presence of gas in the Durham tubes within 24 hours of incubation at 44.5°C were considered positive evidence of fecal coliforms in water samples. Results have been shown in Table 5. All samples of tap water showed positive EC medium test results except sample 08.

Table: 5 *Escherichia coli* (EC) medium

| Samples | EC medium |
|---------|-----------|
| 01 | + |
| 02 | + |
| 03 | + |
| 04 | + |
| 05 | + |
| 06 | + |
| 07 | + |
| 08 | - |
| 09 | + |
| 10 | + |

In MF technique, bacterium on the filter was grown into a visible colony of bacteria on the EMB medium with green metallic sheen. Green metallic sheen indicated the presence of *E.coli* in a water sample. All samples of tap water showed the positive result, producing growth with green metallic sheen except sample 08. Results have been shown in Table 6.

Table 6: Membrane Filtration Technique

| Samples | Growth with green metallic sheen |
|---------|----------------------------------|
| 01 | Growth with green metallic sheen |
| 02 | Growth with green metallic sheen |
| 03 | Growth with green metallic sheen |
| 04 | Growth with green metallic sheen |
| 05 | Growth with green metallic sheen |
| 06 | Growth with green metallic sheen |
| 07 | Growth with green metallic sheen |
| 08 | No growth |
| 09 | Growth with green metallic sheen |
| 10 | Growth with green metallic sheen |

4 Discussion

Bacteriological analysis of tap water samples indicated fecal coliforms in water except sample 08 which was bottle water. The coliforms are the most common group of indicator organisms used in water quality monitoring. These organisms are representative of bacteria normally present in the intestinal tract of mammals including human. Moreover, the presence of coliforms in drinking water could also indicate a breakdown of the treatment process and the transportation of water does not contribute significantly. Such contamination obviously occurs during storage in the house reservoir.

From the results recorded in purified bottle water sample, faecal coliforms were not detected. The bacteriological examination of water sources carried out in this study showed that high total coliforms in tap water of Karachi, Pakistan is indication of recent faecal pollution from human or animal excreta, which may reflect the possibility of potential health hazards. The primary risk of consuming untreated water is the transmission of communicable diseases by pathogenic organisms. Those present in aquatic environments can be of natural origin or may be discharged by humans and other warm-blooded animals. However, the water, which is not suitable for drinking, may be usable for irrigation or for other domestic purposes.

Based on the above assessments bottled water may be of good quality. Water sources such as rivers, wells and surface water cannot be ignored by local water authorities. They should consider a proper regular monitoring programmed to determine the primary sources of contamination, their contribution, health threat, and geographic distribution. In addition, they ought to make recommendations and to develop appropriate control measures to avoid any sudden public health risk from such a vital water source.

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