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Bacteriological Analysis of Water Collected from Different Dams of Rawalpindi / Islamabad Region in Pakistan

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Abstract: In present study, drinking surface water of different dams, located in Rawalpindi / Islamabad region, was analyzed. Water samples were observed bacteriologically for total viable count and total *coliform* count by heterotrophic plate count and MPN method. Pathogenic bacteria were isolated from water samples collected from different dams and related filtration plants and identified by different bio-chemical tests. Water samples contain different pathogenic bacteria like *Bacillus cereus, E. aerogens, S. aureus, E. coli, Salmonella spp., Shigella spp. and Streptobacillus spp.* In all filtration plants, chlorine, a sterilizing agent and the main co-agulant, Alum sulphate, was used. Growth of *Coliforms* was observed after chlorination in water samples, collected from Rawal dam filtration plant. While no growth of any microorganisms was observed after chlorination in case of Simly dam and Khanpur dam filtration plants but water sample taken from storage of Khanpur filtration plant after filtration and chlorination, was also contaminated. The objective of this study was to check the quality of drinking water and comparative analysis of different dams (Rawal, Simly and Khanpur) and related filtration plants which supplying water for drinking and other purposes to different inhabitants of Rawalpindi and Islamabad after filtration and chlorination.

Key words: Pathogenic bacteria, rawal dam, simly dam, khanpur dam, drinking water

INTRODUCTION

Without water there is no concept of life as it is the basic requirement of almost all living organisms. If water is contaminated then it may cause direct danger to health. So the purity and contamination of water is one of the major problems throughout the world. As water can carry number of different organisms to a large number of consumers and over wide areas, the early recognition of the relevant contamination must be ensured[1]. So it is necessity of healthy life that the reservoir, which supply the water for drinking purpose must be checked, whether they are supplying the pure and contamination free water or not.

Water taken from rivers, streams and other different reservoir may look clean and have no undesirable odor or taste. Unfortunately, however, pathogens found in water not only are harmful but also are invisible to the naked eye and may be odorless and tasteless. These bacteria can cause more serious illnesses, such as severe diarrhea, hepatitis or typhoid fever. Water taken from different reservoirs should always be free of contamination or disinfected before being used for drinking or cooking[2]. Microbiological contamination is the primary cause of disease outbreaks with drinking water. The transmission of disease through drinking water is one of the primary concerns for a safe water supply. Different diseases like cholera, typhoid, dysentery and hepatitis have been linked to drinking water contaminated by human waste[3].

All water born parasites originate from human or animal fecal contamination of the environment. Numerous surface water areas such as rivers, lakes and ponds have been found to harbor encysted stages of parasites. Water treatment facilities have reduced the opportunities for transmission of parasites by water. The quality of surface water sources used for drinking water supply varies broadly ranging from good in one area to extremely poor in others. Ideally, sources water should be free of microbial contamination from human and animal activities, free of toxic natural and synthetic chemical contaminates. Water born outbreaks are caused by many different pathogens and each required different method of preservation for example typhoid fever and cholera are water borne diseases. Chlorination and protection of water sources from sewage contamination are effective in preventing transmission of these diseases by water. There are also other methods for reduction of bacteria like filtration of surface water and disinfections of ground

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water. Some bacterial pathogens are common in raw water sources; however, if purification system remains functional, traditional drinking water treatment method will be affective to kill the pathogens[4].

A source water's microbial quality is extremely variable and depends on many factors, including domestic and feral animal activity on the watershed; human activities on the watershed, including recreational, manufacturing and fabrication and agricultural activities; municipal pollution inputs from the raw sewage to primary and secondary wastewater treatment plant effluents; and storm events over the watershed that wash natural and synthetic contaminants into the surface water or that percolate into the groundwater aquifers. Microbial contaminants associated with these sources include bacteria, viruses etc. in both pathogenic and non-pathogenic forms[4].

Rawal dam is situated in Islamabad, is one of the largest unit, which supply water to different sectors of Islambad and Rawalpindi. The main sources of water, which enter in the storage, is from, Q. A. U Nala, Chang Kas, which is coming from Murree and rain water. Khan Pure dam is another largest unit that supplies water to Rawalpindi/ Islamabad regions. It was established in 2000. Its gross storage capacity is about 10700 AF. It is located in N.W.F.P (Hari Pure) and the main sources of water, which enter in the storage, is from Haro River, Murree, Nathiagalli, Ghoragalli and rainwater. Like Rawal and Khanpur Dam, Simly Dam is also the largest reservoir, which supplies water to different sectors of Islamabad. Simly dam was established in 1954 and has total depth of 2300 feet. The main sources from which water comes to simly dam are Murree nala and rainwater. The main supply of Simly Dam is to different sactors of Islamabad for drinking and household purpose but this supply was done after treatment through a filtration plant. Simly Dam filtration plant was established in Jan 1969. It has capacity to supply 24 M.G.D (million gallon per day) filtered water. The main co-agulant, which is used in Simly Dam filtration plant, is Alum sulphate and sterilizing agent is chlorine.

People living in our country have always been facing problem for safe drinking water. In many places, this problem is made harder by the fact that many of the available water sources are unpotable without prior to treatment and even the most common treatments are not available to most of the local population. Numbers of people surfing with common diseases like diarrhea etc. are major causes of mortality. The importance of potable drinking water is therefore obvious.

In this study, water samples were collected from these three different largest reservoirs and an attempt was made to check the quality of water and any kind of bacteria, which may be pathogenic to human life. So water samples of different locations of Simly Dam, Khanpur Dam and Rawal Dam as well as different steps of filtration plant of Simly Dam, Khanpur Dam and Rawal Dam were checked for complete bacteriological analysis.

MATERIALS AND METHODS

Sampling Methodology: Water samples of three different dams (Rawal, Simly and Khanpur Dam) and related filtration plant, were collected in sterile leak proof glass bottles following the standard method of water collection for microbiological analysis between August and October 2002, from different points. Seven water samples were collected from Rawal Dam and related filtration plant. Seven water samples were collected from simly Dam including three samples from different locations of main storage of Simly Dam and four samples from Simly Dam filtration plant. Six water samples from main storage of Khanpur Dam and related filtration plant (Table 1).

Cultured Media: All the chemicals used were of analytical grade and were obtained either from E. Merck or from Sigma chemical company. Culture media were purchased from Oxoid Limited.

Biochemical Tests: All water samples, which were collected in sterile bottles, were examined for different pathogenic bacteria with in 24 h after collection. Water samples were tested for Standard Plate Count (SPC) and Coliform Count (MPN), by serial dilution on the nutrient and by Most Probable Number technique[5].

Table 1: Samples Collected from Different Dams

Rawal Dam	Simly Dam	Khanpur Dam
Chang Kas (Coming from Chattar)	Raw water	Open canal water
Q.A.U Nala (Coming from Q.A.U)	After sedimentation	Main storage
Raw water	After filtration	Raw water (Filtration plant)
After sedimentation	After chlorination	After sedimentation
After filtration	Three samples from	Afterchlorination
After chlorination	different locations of main storage.	Storage tank (After filtration + chlorination
Simply filtered water (Supplied to mosque)		

Only desired colonies were picked up by sterile platinum loop and sub culturing was continued until the pure culture was obtained. Purification of the culture was confirmed by Gram staining. Pure colonies were again cultured on specific media agar plates and broth and stored at 4°C in refrigerator until used. After isolation of different pathogenic bacteria, different biochemical tests were performed for identification of these pathogenic

bacteria after obtaining the pure culture. Purification of culture was confirmed by Gram staining and different tests like Catalase test[6] NaCl tolerance test[5] Acidification of sugars[7], citrate utilization test[5], temperature tolerance test[5], H_2S production[5] and confirmed test for *E.coli*[8] were performed for identification of pathogenic bacteria.

RESULTS AND DISCUSSION

These water samples were brought to the microbiology / analytical laboratory, for microbiological analysis. Water samples were tested for heterotrophic plate count by serial dilution on nutrient agar and for total Coliform by MPN technique. Results of total viable count and total Coliform (Table 2 and 3) showed that in case of Rawal Dam water, first three water samples (Chang Kas, Q.A.U Nala, Raw water of filtration plant) were found to be highly contaminated with pathogenic bacteria and these might be able to cause serious danger to health, while rest of water samples (after sedimentation, after filtration) number of micro-organisms were reduced and after filtration, number of bacteria were less than after sedimentation. 110 MPN/100ml microorganisms (Total Coliform) were observed in the water samples, which were collected after chlorination but water supplied to Mosque after filtration was also contaminated.

In case of Khanpur dam water samples, first four samples were found to be highly contaminated but the numbers of microorganisms were reduced after sedimentation. No growth of any kind of organisms was observed in the sample which was taken after chlorination but the sample taken from storage tank after filtration and chlorination was found to be contaminated with 21 MPN /100 ml of Coliform

In case of Simly dam water samples, all the water samples were found to be contaminated except after chlorination, while rest of water samples (after sedimentation, after filtration) number of micro-organisms were reduced and after chlorination no growth of microorganisms was observed.

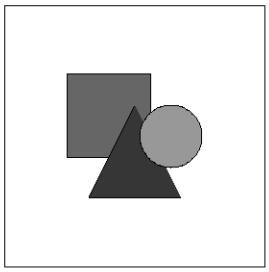

Fig. 1: Growth of *Baccilus cereus* Var. mycoides. on media plate

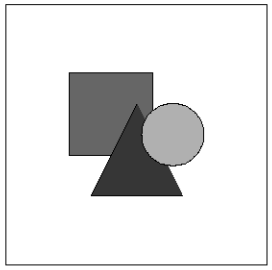

Fig. 2: Microscopic representation of *E.aerogens*

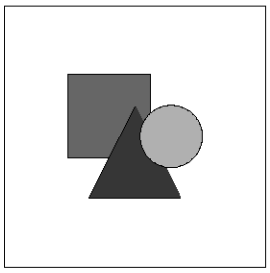

Fig. 3: Growth of *E.coli* on media plate

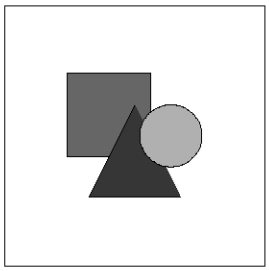

Fig. 4: Microscopic representation of *Staphylococcus aureus*

Table: 2 Total bacterial count

Rawal Dam		Simly Dam		Khanpur Dam	
Samples (locations)	Total viable count (TVC) per ml	Total viable count Samples (locations)	(TVC) per ml	Total viable count Samples (locations)	(TVC) per ml
Chang Kas	6×10^4	Main storage (East)	18×10^2	Open canal water	7×10^3
Q. A. U Nala	2×10^4	Main storage (West)	14×10^3	Main storage	3×10^3
Raw water	15×10^4	Main storage (centre)	16×10^2	Raw water	52×10^3
After Sedimentation	3×10^2	Raw water	89×10^3	After Sedimentation	3×10^3
After Filtration	ND	After Sedimentation	21×10^2	After Chlorination	ND
After Chlorination	2×10^2	After Filtration	27×10^3	Storage Tank (filtered	ND
End source (only filtered to mosque)	2×10^3	After Chlorination	ND	+Chlorinated)	

Table: 3 Total *Coliform* count MPN values 100^{-1} ml of samples and 95 % confidence limit for various combinations of positive and negative results (when five 10 ml, five 1 ml and five 0.1 ml test portion was used)

Samples (locations)	No. of tubes giving a positive reaction 5 of 10 ml	5 of 1 ml	5 of 0.1 ml	MPN (PER 100 ml)	95% confidence limit Lower	Higher
Rawal Dam						
Chang Kas	5	5	3	920	300	3200
Q. A. U Nala	5	4	2	220	57	700
Raw water	5	4	0	130	35	300
After Sedimentation	0	0	0	<2	<1	7
After Filtration	0	0	0	<2	1	7
After Chlorination	5	3	1	110	31	250
End source (filtered)	5	3	3	180	44	500
Simly Dam						
Main storage (East)	5	2	2	94	28	220
Main storage (West)	5	3	0	79	25	190
Main storage (centre)	5	2	1	70	23	170
Raw water	5	3	3	180	44	500
After Sedimentation	5	3	2	140	37	340
After Filtration	5	3	1	110	31	250
After Chlorination	0	0	0	<2	<1	7
Khan pur Dam						
Open canal water	5	4	2	220	57	700
Main storage	5	3	2	140	37	340
Raw water	5	4	1	170	43	490
After Sedimentation	5	2	1	70	23	170
After Chlorination	0	0	0	<2	<1	7
Storage Tank (filtered + Chlorinated)	4	1	1	21	7	63

MPN = Most Probable Number

The bacteria, which were isolated from samples during analysis, include *Bacillus cereus, E. aerogens, S. aureus, E. coli, Salmonella spp., Shigella spp.* and *Streptobacillus spp.*. Bacteria, which were isolated, along with their bio-chemical properties, are given in Table 4. All the strains isolated were catalase positive and Gram negative except *Staphylococcus aureus* and *B. cereus.* Citrate was utilized as a carbon source by *Salmonella spp, Staphlococcus aureus and Enterobacter aerogens,* while all other were citrate negative and *Bacillus cereus* showed late citrate positive. Different concentrations of NaCl tolerance was observed, all the strains grew at 4% NaCl but growth of *Enterobacter*, *Staphylococcus aureus, Bacillus cerus* and *Streptobacillus spp.* was also observed at 6.5% NaCl containing media. Different bacteria have the different ability to grow at different temperature. Results are given in the table 4. Acid production from different sugars was also checked these bacteria, results of fermentation tests is given in table-5. The results were similar to the Bergey's manual of determinative bacteriology[9] and Methods for examination of water and food by American Public Health Association[5].

All the water samples collected from Rawal dam were contaminated. Raw water was highly contaminated with *E.coli, Bacillus cereus, Staphylococcus aureus* and *Coliform.* Numbers of microorganisms were decreased after sedimentation and no microorganisms were found after filtration but only growth of *Coliform* was found after chlorination. It may be due to unhygienic conditions of storage tank, where water was stored. It is cleared from the above discussion and results that water, which was supplied from Rawal dam filtration plant, was not fully satisfied and potable drinking water. So urgent attention is required at this point because this water is supplied to a large community of Islamabad and it may cause a serious danger to their health.

Simly Dam supplies water to different sector of Islamabad was fit for drinking purpose because after chlorination no growth of microorganisms was observed.

Table: 4 Biochemical characteristics of bacteria isolated from different dams

Properties	*E. coli*	*Salmonella spp.*	*Streptobacillus spp.*	*Shigella spp.*	*E. aerogens*	*S. aures*	*B. cereus* Var.mycoides
Morphology	Rods, Single, Pairs	Rods, Single, Pairs	Rods, Long Chains	Rods, Single, Pairs	Rods, Single, Pairs	Spherical clusters	Rods, Single, Pairs
Motility	-	+	-	-	-	-	-
Growth at 10 °C	-	-	-	-	+	+	+
Growth at 37 °C	+	+	+	+	+	+	+
Catalase Test	+	+	+	+	+	+	+
Gram's staining	-	-	-	-	-	+	+
Growth in 4% NaCl	+	+	+	+	+	+	+
Growth in 6.5% NaCl	-	v	+	-	+	+	+
Citrate utilization	-	+	-	-	+	+	x
H_2S Production	-	+	-	-	-	-	-

Table: 5 Results of sugar tests for identification

Strains	Fruc.	Gluc.	Galac.	Suc.	Mal.	Mani.	Lac.	Arab.	Xyl.	Dulci.
E. coli	+	+	+	-	+	+	-	+	+	+
Salmonella spp.	v	v	v	-	+	+	+	+	+	+
Streptobacillus spp.	+	+	+	-	+	-	-	-	-	-
Shigella spp.	+	+	+	v	+	+	+	+	-	+
E. aerogens	v	+	v	+	+	+	-	+	+	-
S. aures	+	-	+	+	+	+	+	-	-	+
B. cereus	+	+	-	+	-	-	-	-	-	-

+ = Positive reaction - = Negative reaction v = Variable reaction x = Late positive

Raw water was found to be highly contaminated with pathogenic bacteria but number of microorganisms reduced after sedimentation and after filtration number of microorganisms were suddenly increased which were destroyed by the chlorination. So there is a need to check the filtration plant and filter should be replaced or checked for the best results

In case of Khanpur dam, less growth of microorganisms was observed as compared to Simly and Rawal dam and these numbers of microorganisms were reduced at each step of treatment. Water samples collected from storage tanks were found to be contaminated and water collected from the supply pipe after chlorination was free of contamination and totally fit for drinking purpose, so in comparison with Rawal dam, Simly dam & Khanpur water supply system was better.

Recommendations: Amount of chlorine at the point of chlorination should be checked and storage reservoir should be checked after proper time interval or by removing source of pollutions.

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