

Physical and Microbiological Assessment of Drinking Water of Nomal Valley, Northern Areas, Pakistan

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Abstract.- Drinking water samples collected from source, inlet, outlet of the water reservoirs and from the water supply distribution of Gilgit was found contaminated with thermophilic coliforms throughout the year except at the source in January and February. This contamination was highest at source from May to August (90 – 280 fecal coliforms /100 ml water) and it increased from source to inlet of the water reservoir (195 – 434 fecal coliforms /100 ml water). Inside the water reservoir there was no treatment of water and the contamination slightly increased at outlet (202 - 437 fecal coliforms /100 ml water). Turbidity of water started from March, was 2000 TUs in June and <5 TUs at the end of November. The turbidity slightly decreased from source to inlet of the water reservoir and there was no change in distribution. The temperature varied from 6 to 7.34°C during Winter and Autumn, 7 to 8.67°C during Spring, while it was 11 to 14.34°C in Summer. Most of the strains were sensitive to Ciprofloxacin and all were resistant to Vancomycin and Erythromycin.

Key words: Fecal coliforms, drinking water, antibiotic resistance.

INTRODUCTION

Northern Area is situated at 1500 – 8000 meters above the sea level and covered by high mountains and these mountains separate one area from the other and each area have its own water source. Nomal valley is at 30 kilometers from center Gilgit. The climate is intensely cold in winter, with heavy snow fall. In spring and summer seasons when the temperature becomes high this snow and glaciers melts and water flow towards the inhabited area. The water reservoir is in the middle of the village and is fed by a five Kilometer long open channel. This surface water during its flow it carries clay and by contact of human and other animals it becomes polluted by bacteria, viruses, protozoan and helminthes parasites. Bacteriological contamination of drinking water has been reported to be one of the most serious problem throughout the country in rural as well as urban areas (Abid and Jamil, 2005; Kahlowan *et al.*, 2004; Sun-Ok *et al.*, 2001). In Pakistan water related diseases (diarrhoea) is commonest ailment which is encountered almost by every individual at least once a year (Hafiz *et al.*, 1991; Karamat *et al.*, 1993), and 30-50% of hospital

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admission are due to diarrhea (Imran, 1978, Haque and Ali, 1986). A high degree of water contamination was found in setting where the incidence of diarrhea was high (Black *et al.*, 1989; Henry *et al.*, 1990; Molback *et al.*, 1989). The impact of safe drinking water supply and sanitation facilities can help in preventing water and sanitation related diseases (Boot and Cairncross, 1993; Gorter *et al.*, 1997).

There are a range of health issues related to microbiological contamination that arise from contaminated drinking water quality (Fewtrell *et al.*, 2001; Redi *et al.*, 2002). The organisms commonly used as the indicator of fecal contamination are certain commensal intestinal bacteria of animals especially *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens* (Muneer *et al.*, 2001; Mcfeters *et al.*, 1995; Fiksdal *et al.*, 1994; Szewzyk *et al.*, 1994; Elmund *et al.*, 1999) as they are always present in the feaces of man and warm-blooded animals intestine, their presence in drinking water indicates fecal contamination (Lechevalliet *et al.*, 1990; Szewzyk *et al.*, 1994). Coliform bacteria may not cause disease (Holmes and Nicolls, 1995). According to WHO (1971) the drinking water is safe if it does not contain any coliform bacteria in

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100 ml sample (Pillai *et al.*, 1999).

This study may generate some data on the physical and microbiological quality of drinking water of a representative valley in Northern Areas of Pakistan.

MATERIALS AND METHODS

Sample locations

Drinking water samples were collected each month from 8th April 2006 to 10th April 2007, from the main source (nallah: big streams flowing from top to bottom of the hills) about one hundred meters away from the inhabited area, inlet, outlet of water reservoir (open water tank) and from the distribution of water supply system and processed *in-situ*.

Collection of water samples

Water samples were collected by using the WHO guidelines (1984). Samples from the source and inlet of the water reservoir were collected in the cup of the Del-Agua water testing kit (Fig. 1). Cup was sterilized and sample was taken from the mid of the water. For collection of water sample from the outlet of the water reservoir and from the distribution system the nozzle of the tap was cleaned and sterilized by flaming. The tap was allowed to cool by running the water for 2 minutes and then taken the sample in sterilized cup and processed *in-situ*.



Fig. 1. Del-Agua water testing kit used for water testing.

Media

Commercially available Membrane lauryl sulphate broth (Oxoid) was used for isolation of fecal coliform bacteria. The broth was prepared by mixing 7.62 gram in 100 ml distill water and autoclaved at 121°C for 15 minutes.

Commercially available sensitest agar (Oxoid) was prepared by suspending 2.3 gram of dehydrated agar (Oxoid) in 100 ml distill water and sterilized by autoclaving at 121°C for 20 minutes and cooled to 50 °C and poured into sterilized glass plates (90 mm) and solidified at room temperature.

Filtration of water through the membrane

The filtration funnel containing the bronze disc was sterilized by methanol fuming and the funnel was fitted on the vacuum cup. The filter funnel was opened and sterilized filter membrane (Gelman Sciences) (0.45 µm pore size) was placed on the bronze disc filter support. The funnel was refitted and the required volume of water was followed by creating vacuum with the vacuum pump. After filtration the membrane was transferred carefully with the help of sterilized tweezer to the absorbent pad (GELMAN) already saturated with sterilized Membrane lauryl sulphate broth and incubated at 44°C for 18 hours. The characteristic of colonies were read and counted on the membrane in good light with hand lens.

In December, January, February March and April 100 ml of water was filtered through the filter membrane and in May, June, July, August and September 50 ml water was filtered due to its high turbidity and colony count. The 50 ml sample was diluted by adding already sterilized transparent 50 ml water and calculated the results out of 100 ml.

Sensitivity test

Antibiotic susceptibility of the isolated bacteria was tested by disc diffusion method (Bauer *et al.*, 1966) against Ciprofloxacin (5 µg per disc), Amoxicillin (30 µg), Cefazidime Acid (30 µg), Nalidixic Acid (30 µg), Vancomycin (30 µg), Cefaclor (30 µg) and Erythromycin (10 µg).

Measurement of turbidity and temperature

Turbidity of water was measured by using the nephelometric turbidity tube covering the range 5 to 2,000 TUs.

Temperature of water was measured by mercury thermometer in °C.

RESULTS

Monthly colony count of drinking water at different sources

Table I shows month wise variation in the incidence of fecal coliforms contamination in drinking water of Nomal valley at its different sampling points. In the month of January and February there was no fecal coliform contamination in the inlet, at the source whereas, there were only 4-5 colonies/100ml in the outlet of the water reservoir and distribution. In the months of March and April there was only one fecal coliform colony at source and this contamination greaterly increased in the inlet of the water reservoir (38- 41 colonies/100 ml). In the outlet of the water reservoir this contamination slightly increased (43-48 colonies/100ml) and further increased (51-55 colonies /100ml) in the distribution. In the months from May to September the water was highly contaminated (90-280 colonies /100 ml) at source and contamination increased (90 -461 colonies /100) in inlet of the water reservoir. This contamination was further increased (202 – 531 colonies /100 ml) in the outlet of the water reservoir. In the distribution, the contamination further increased (223-554 colonies /100 ml). The contamination level decreased in the months of October to December (10 -20 colonies /100 ml) at source. In the Inlet of water reservoir (28 – 112 colonies /100 ml), outlet (29 – 114 colonies /100 ml) and in the distribution (32 – 135 colonies /100 ml).

Seasonal colony count of drinking water

Table II shows seasonal variation of fecal coliforms contamination at different water sampling points. At the source the highest contamination was found in the summer with 580 fecal coliforms/100 ml followed by spring with 92 colonies /100 ml, autumn with 45 colonies /100 ml and in the winter 15 colonies of fecal coliforms.

Monthly turbidity variation in the drinking water

Table III shows monthly turbidity levels at all the sampling points at source, inlet, outlet of the

Table I. Month-wise colony count of fecal coliforms /100ml drinking water from different sampling points of Nomal Valley.

Months	Source	Inlet water reservoir	Outlet water reservoir	Distribution
January	0	4	4	5
February	0	5	5	6
March	1	41	43	51
April	1	38	48	55
May	90	195	202	223
June	100	262	267	292
July	200	461	531	554
August	280	434	437	466
September	15	275	302	314
October	10	62	61	72
November	20	112	114	135
December	15	28	29	32

Table II. Season-wise colony count of fecal coliforms /100ml drinking water from different sampling points of Nomal Valley

Seasons	Source	Inlet water reservoir	Outlet water reservoir	Distribution
Spring	92	91	98	110
Summer	580	386	412	437
Autumn	45	150	159	174
Winter	15	12	13	15

Table III. Month-wise variation of turbidity of drinking water at different sampling points of Nomal valley.

Months	Source	Inlet water reservoir	Outlet water reservoir	Distribution
January	0	0	0	0
February	0	0	0	0
March	30	26	26	26
April	300	262	262	262
May	500	425	425	425
June	2000	1625	1625	1625
July	1000	812	812	812
August	200	162	162	162

September	50	46	46	46
October	20	20	20	20
November	0	0	0	0
December	0	0	0	0

water reservoir and in distribution. At all the sampling points there was no turbidity in the months of January and February. At source, the turbidity started from March (30 TUs), decreased in the inlet of the reservoir (26 TUs), while there was no change of turbidity in the outlet and distribution. At source the turbidity highly increased from April (300 TUs.), May (500 TUs), June (2000 TUs) and it also decreased in the inlet of the water reservoir (262 TUs., 425 TUs., 1625 TUs. respectively) and again there was no change in the turbidity in the outlet and distribution system. The turbidity started to decrease gradually at source in the months of July (1000 TUs), August (200 TUs) and September (50 TUs). It decreased to 812 TUs, 162 TUs, 46 TUs in the inlet of the reservoir during these three months, respectively. Again there was no change in the outlet and distribution. In the month of October the turbidity at source was 20 TUs and it remained the same at all the sampling points. In November and December there was no turbidity in water (<5 TUs).

Seasonal variation of turbidity in drinking water

Table IV shows seasonal variation of turbidity at different sampling points. At source the highest turbidity was found in summer (3200 TUs) and this turbidity decreased in the inlet of the water reservoir ((24 TUs). There was no change in turbidity in the outlet of the water reservoir and in the distribution system. In spring the turbidity was 830 TUs at source and in the inlet it decreased to 24 TUs, while in outlet and distribution there is no change in turbidity. In autumn the turbidity at source decreased to 70 TUs and in inlet it decreased to 22 TUs. There was no change in turbidity at other sampling points. In winter the turbidity was < 5 TUs at all the sampling points.

Antibiotic sensitivity

Table V shows antibiotic resistance of *Escherichia coli* against Ciprofloxacin, Amoxicillin, Ceftazidime, Nalidixic acid, Vancomycin, Cefaclor and Erythromycin. The highest number of strains were sensitive to Ciprofloxacin 20

(95.23%), Ceftazidime 17 (80.95%) and Cefaclor 11 (52.38%). With Nalidixic Acid and Amoxycillin the sensitivity was 5 (23.80%) and 3 (14.28%), respectively.

Table IV.- Season-wise variation of turbidity of drinking water at different sampling points of Nomal valley.

Seasons	Source	Inlet water reservoir	Outlet water reservoir	Distribution
Spring	830	24	24	24
Summer	3200	867	867	867
Autumn	70	22	22	22
Winter	0	0	0	0

Table V. Antibiotic sensitivity of *Escherichia coli* isolated from drinking water.

Name of antibiotics	Total No. of strains investigated	No. of strains sensitive	Percentage sensitivity
Ciprofloxacin (5 µg)	21	20	95.23
Amoxicillin (10 µg)	21	03	14.28
Ceftazidime (30 µg)	21	17	80.95
Nalidixic Acid (30 µg)	21	05	23.80
Vancomycin (30 µg)	21	00	00.00
Cefaclor (30 µg)	21	11	52.38
Erythromycin (10µg)	21	00	00.00

Temperature of drinking water

Table VI shows the temperature variability in each month. In the first four months the temperature at source was 6°C and it increased 1°C at inlet of the water reservoir and there was no variation in the outlet and distribution system. In the months of April and May the temperature at source was 10 and 12°C, respectively and it also increased 1°C at the inlet and there was no change in the outlet of the water reservoir. There was an increase of 1°C in the distribution system. The temperature was higher in the months of May, June and July *i.e.* 8, 10, 12°C, respectively and that increased 1°C in May and 2°C in June and July in the inlet of the

water reservoir. There was no change in the temperature in the outlet of the water reservoir. In distribution system the temperature increased 1°C in May and 2°C in June and July. From August onward the temperature started to decrease *i.e.* 11°C at source and in the inlet of the water it was 12°C. No change was observed in the outlet of the water reservoir. It increased 1°C in the distribution system. In September the temperature was the same at all the sampling points as in May. In October, November and December the temperature was the same at source, Inlet and outlet as in the months of January, February and March, while in the distribution it increased by 1°C.

Table VI. Month-wise variation of temperature of drinking water at different sampling points of Nomal valley.

Months	Source	Inlet water reservoir	Outlet water reservoir	Distribution
January	6	7	7	7
February	6	7	7	7
March	6	7	7	7
April	7	8	8	9
May	8	9	9	10
June	10	12	12	14
July	12	14	14	16
August	11	12	12	13
September	8	9	9	10
October	6	7	7	8
November	6	7	7	8
December	6	7	7	8

Table VII. Season-wise variation of temperature of drinking water at different sampling points of Nomal valley.

Seasons	Source	Inlet water reservoir	Outlet water reservoir	Distribution
Spring	7	8	8	8.67
Summer	11	12.67	12.67	14.34
Autumn	6	7	7	7.34
Winter	6	7	7	7.34

Table VII shows the seasonal variation of temperature during the year. In the Spring the temperature of water at source, inlet, outlet and distribution was 7, 8, 8, and 8.67°C respectively, In

Summer the temperature was highest *i.e.* 11, 12.67, 12.67 and 14.34°C at source, inlet, outlet and distribution system. In Autumn and winter it is 6, 7, 7 and 7.34°C at all the sampling points.

DISCUSSION

The microbiological results obtained by this study reveal that the water supply system is very simple *i.e.* just storage of water and distribution to facilitate the community to easy asses of drinking water .

In the month-wise sampling from January to April the fecal coliform contamination, turbidity and temperature is very low at source (January and February <5 TUs) and in inlet of the water reservoir the contamination increased very slightly and there is decrease of turbidity and temperature. In the outlet and distribution there is very slight increase in contamination and there is no variation in turbidity, because the water is not stored in the water reservoir for longer period.

In the months (May – August) the detection of fecal contamination was very high even at source (90 – 280 fecal coliforms/100 ml water) and it gradually increased in the inlet and outlet of the water reservoir and in its distribution. During these months the turbidity (200 – 2000 TUs) was also very high at source and it decreased during its flow from the source to inlet of the water reservoir and there was no variation in the outlet and distribution system. The temperature was also high at source and it became high from source to inlet of the water reservoir. This high level of contamination is due to increase in temperature and even in nallah it is inhabited and people are more actively related with agriculture and washing of their cloths. Same type of results had been received by Muneer *et al.* (2002) at University of the Punjab Campus. The occurrence of coli form bacteria was significantly higher when water temperature were >15°C (Lechevallier, 1996). The increased in temperature may be due to the increased in the level water it carry clay and sand particles.

In seasonal assessment the highest level of fecal coliform contamination and turbidity was detected in summer. This is also due to high level of activities of agriculture and movements of livestock. A summary of report from different regions of the

world showed that maximum number of bacteria were detected during the summer months (Iriberry *et al.*, 1987). Ahmed *et al.* (2002) reported in their study many cases of cholerae from this valley in summer seasons.

Our results also show that in the distribution system there is no increase in the fecal coliform contamination. The plumbing system is new and working properly. The age of plumbing system was also found to have impact on the water quality (Augoustinos *et al.*, 1995)

On the bases of above study it is recommended that the water should be treated during summer seasons and let the water to be stored for longer period so by sedimentation the turbidity may decrease.

The sensitivity of the organisms shows that very frequently used antibiotics developed resistance.

The availability of safe drinking water is a big problem throughout the world. Pakistan's population has a current water supply coverage of 79% (Govt. of Pakistan 2000) this inadequate supply also poses health risks to the consumers because of its poor quality. Augoustinos *et al.* (1995) collected water samples from private houses and apartment buildings in South Africa isolated *E. coli*, *Acinetobacter*, *Klebsiella* and *Citrobacter* in 33 per cent specimens. In Pakistan (Ahmed, 1994) reported the microbiological quality of drinking water from some important lakes (Indus at Kotri 150-400 fecal coliform /100ml, chenab at Rakh branch canal 1050-5000 fecal coliform /100ml, River Ravi at Balloki 1200-15000 fecal coliform /100ml) which are used as raw water sources for drinking supplies.

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