RESEARCH PAPER

Microbiological quality assessment of drinking water in three selected areas of district Mansehra

Qazi Inam ul Haq[1], Mahrukh Naseem[2], Nayab Khan[2*], Muhammad Adnan khan[3], Syed Umair Ahmad[3], Syed Qasim Shah[4], Sabir Shah[5]

[1]*Department of Microbiology, Hazara University Mansehra, Pakistan*

[2]*Department of Zoology University of Balochistan Quetta, Pakistan*

[3]*Department of Bioinformatics, Hazara University Mansehra, Pakistan*

[4]*Govt. Boys Inter College Nawa Killi Quetta, Pakistan*

[5]*Bolan Medical and health sciences university Quetta, Pakistan*

Abstract

Water has a great importance in our life, primary used for drinking purpose but due to microbial contamination, it becomes unfit for drinking. The present study was conducted to examine the bacterial contamination and its count in three water sources i.e. wells, tube wells and hand pumps in three selected areas of district Mansehra, i.e. Mansehra city, Union Council Dhodial and Union Council Shinkiari. A total of 64 samples of water were analyzed for Total Plate Count (TPC), Coliform, Fecal coliform and *E. coli*. Techniques like multiple tube fermentation, total plate count and latex test reagent kit was used for bacterial conformation. (45/64) water samples were analyzed from the wells of Mansehra city, Union Council Dhodial and Union Council Shinkiari. Only four and three water samples were analyzed for bacterial load from tube wells water of Mansehra city and Union Council Dhodial. From hand pump water three, four and five samples (total 12) were examined from Union Council Dhodial, Mansehra city and Union Council Shinkiari respectively. The samples were analyzed for microbial parameters and found that more than 70% water samples were contaminated with fecal coliform including *Escherichia. coli*. Present study indicates that there is lack of cent per cent purity in any water source from study area i.e. Mansehra district. Hence it is concluded that the water in study areas was contaminated and unfit for drinking purposes according to Pakistan standards.

* **Corresponding Author:** Nayab Khan ✉ nayabkhan1919@yahoo.com

Introduction

Water is an imperative natural resource that it is used for many purposes; it plays vital role in regulating body temperature, digestion of food and removal of body wastes (Behman, 1996).The physiological diverse properties of water have been used in making traditional and modern medicine, in all forms of hydrotherapy (Mac Donnell, 1996). Without it transport of various substances within the body cannot take place. The existence of water in body tissues helps in defending the body against infections and shocks (Obi *et al.,* 2002). The spinal cord, eyes and brain are among the sensitive structures that need a shielding layer of water. As a liquid, water possesses such splendid physical and chemical properties that outclass all other liquids of the earth. Because of these unique properties water is suitably fit for the exercise of life on earth (John, 1991). The quality of drinking water can be examined in a number of ways. It can be tested for the presence of bacteria and other microorganisms, for the levels of minerals that occur naturally in water and for other physical and chemical factors (Edberg *et al.,* 2000). For usage, as a healthful fluid for human consumption, water must be free from pathogens, from high levels of minerals, any color, and smell and from disagreeable taste (WHO, 2011).

The hospital data reflect that numerous diseases treated are caused by waterborne microbes suggesting that a significant proportion of morbidity in Pakistan is due to usage of contaminated water e.g. diarrhea resulting from gastrointestinal infections(Bashir *et al.,*1999). World Health Organization (WHO) recommended the following parameter to determine the hygienic quality of water: (a) *Escherichia coli* and thermo-tolerant Coliforms accepted as suitable alternatives, (b) chlorine residual (if chlorination is practiced), (c) pH and (d) turbidity (WHO, 2003). The major objective of the proposed was to analyze different water samples qualitatively and quantitatively in three water sources used for domestic purposes in district Mansehra of KPK province. The study was conducted in the most populated district of Hazara division, i.e. Mansehra district. The major objectives of the study are:

First, to find out the bacteriological contamination in different water samples used for domestic purposes from three selected areas in district Mansehra of KPK province.

Second, to compare the intensity of contamination in drinking water from various sources in three selected areas i.e. Mansehra City, Union Council Dhodial and Union Council Shinkiari.

Third, to create awareness among people about the problems associated with the utilization of contaminated water.

Materials and methods

The present study was directed to check the contamination due to bacteria and its count in three water resources i.e. wells, tube wells and hand pumps in three selected areas of district Mansehra; Mansehra city, Union Council Dhodial and Union Council Shinkiari were selected. A total of 64 water samples were collected during March, 2013 for Total Plate Count (TPC), Coliform, *E. coli* and Fecal coliform. Four, eight and 33 (45) water samples were analyzed from the wells of Mansehra city, Union Council Shinkiari and Union Council Dhodial, respectively. From hand pumps four, three and five (12) water samples were examined from Mansehra city, Union Council Dhodialand Union Council Shinkiari, respectively. No tube well was examined in Union Council Shinkiari area, whereas four and three (7) water samples were collected for bacteriological count from tube wells of Mansehra city and Union Council Dhodial, respectively.

Sample collection

Samples of water (100 ml) from wells, tube wells and hand pumps were collected from Mansehra city, Union Council Dhodial and Union Council Shinkiari areas. All these water samples were analyzed bacteriologically for total plate count, coliform, fecal coliform and *E.coli.* As described in standard

methods, microbiological analyses of water samples were performed for water examination (APHA, 1998).

Determination of Total Plate Count

The total plate count is designed to give the estimate for total number of aerobic organisms in any particular sample. Two drops from water sample were taken with the help of a dropper, and were added to a sterilized Petri dish having Plate Count Agar (PCA) and were gently agitated for some time for thorough mixing. Finally they were incubated for 48 hours at 35 °C and examined for the colonies of bacteria which were counted using a colony counter (APHA, 2001).

Confirmation Test for Fecal Coliform

First, gently agitated each LT tube containing gas and transferred loopful of suspension to tube of EC medium. Second, incubated EC medium tube at 45.5°C for 48 hours. Examined for gas production after 48 hours, if negative again examined after 24 hours. (APHA, 2001).

Confirmation test for Escherichia coli (E.coli)

Streaked loopful of suspension from each Escherichia colimedium tube containing gas on Eosin Methylene Blue (EMB) agar. Incubated plates for 18 to 24 hours at 35°C. Examined plates for typical *E.coli* colonies that is dark centered with or without metallic sheen. The presence of Gram-negative confirmed *E.coli* (APHA, 2001).

Procedure for Quantitative Assessment of Coliform

One ml from the original sample (10 ml water sample) was inoculated into a series of 10 fermentation tubes each containing nine ml of Lactose broth with inverted Durham tube and incubated at 35 ± 0.5°C for 24 - 48 hours. After a period of 24 hours incubation, tubes were looked for any evidence of gas production. In inverted vial gas production was examined. The tubes with negative result were incubated again for 24 hours and again examined for gas production (APHA, 2001).

Procedure for Quantitative Assessment of Fecal Coliform

The MPN of Coliform bacteria was measured using multiple fermentation technique (APHA 2001).The tubes displaying gas were used to inoculate *Escherichia coli* (E.C) broth and these were further incubated for 24 hours at 44.5°C and later examined for gas production (APHA, 2001).

Procedure for E. coli Qualitative Test

Eosine Methylene Blue (EMB) agar was used to detect the presence of *E. coli*. All E.C broth tubes which were positive for gas, were cultured on EMB agar and then they were allowed to incubate at 35°C for a period of 18 to 24 hours. After the period of 24 hours, those tubes that were positive for *E.coli*, were separated for confirmation test. (APHA, 2001).

Multiple Tube Techniques

The particular technique was considered, using a set of ten tubes (one for each sample). Tubes were filled with nine ml medium and one ml water.

These tubes were incubation and then examined for the diagnosis, gas production reaction for Coliform as shown in figure no.1. Most Probable Number was calculated for Coliform and F. Coliform based on proportion of confirmed Lactose broth and *Escherichia coli* medium respectively, out of ten tubes containing gas (APHA, 2001).

Confirmation Tests by Kits

*E.coli*0157:H7 (Pathogenic strain) LATEX TEST REAGENT KIT is an agglutination test kit to confirm identification of *E.coli* serogroup 0157:H7 antigen on laboratory culture media.

Data Analysis

All the laboratory work was performed in Diagnostic Lab of Microbiology Department at Hazara University and National Lab Mansehra. The data were subjected to analysis by MS Excel, for calculating mean and standard deviation etc. and graphical presentations.

Results

Quality of wells water in the three study areas

Mansehra city and union council dhodial water was highly contaminated in term of total plate count.

Table 1. Mean values of TPC / ml of wells water of district Mansehra.

Location	No. of Samples	Mean	Std. Deviation
Mansehra city	4	2382	± 3053.66
Dhodial	33	3046.15	± 2581.98
Shinkiari	8	945.62	± 18128.68

Table 2. Mean and Standard Deviation of the MPN of bacterial count from wells water samples collected from Mansehra District.

Location	Samples	Total	Coliform / ml	F. coliform / ml	*E. coli*
Mansehra City	4	Mean	2.4	2	Present
		Std. Deviation	1.63	1.4	
Shinkiari	8	Mean	9.2	6.4	Present
		Std. Deviation	10	7	
Dhodial	33	Mean	4.04	2.4	Present
		Std. Deviation	3.4	3	

The wells water from the Dhodial area was also unsafe for drinking purposes. The differences among the three mean concentrations were not significant as shown in table no. 1 and 2. It was therefore concluded that the concentrations of F.coliform in well water at the three locations were not significantly different from one another.

Quality of tube wells water in two study areas
Based on the TPC estimates, quality of tube well water from Mansehra city was comparable to that from Union Council Dhodial tube well water as shown in Table3. The mean MPN / ml values of Coliform and F.coliform from the two locations did not vary significantly from one another, the mean values of Mansehra city tube wells water for Coliform and F.coliform / ml were 3 and 1.65 respectively, while the mean values of Union Council Dhodial tube wells water for Coliform and F.coliform / ml were 2.7 and 1.5 respectively.

Table 3. Mean values of TPC/ ml for the tube wells water from Mansehra city and Union Council Dhodial.

Location	No. of samples	Mean	Std. Deviation
Mansehra city	4	3971.75	± 2512.61
Dhodial	3	2066.7	± 1147.05

Table 4. Mean values of TPC/ml of hand pumps water from district Mansehra.

Location	No. of Samples	Mean	Std. Deviation
Mansehra city	4	6014	± 3633.33
Dhodial	3	4512	± 1080.1
Shinkiari	5	2115	± 1126.58

The bacterial load with respect to Coliform and F.coliform in tube wells water at Union Council Dhodial was equal to that at Mansehra city.

Quality of hand pumps water in three study areas

Mansehra city hand pumps water was highly contaminated. After mansehra city, union council Dhodial area was badly affected, shown in table 4 and 5. Figure no. 2 and 3 are showing the mean MPN values for coliform and F.coliform.

Table 5. Mean and Standard Deviation of the MPN of bacterial count from hand pumps water samples collected from different localities.

Location	Samples	Total	Coliform / ml	F.coliform/ ml	*E. coli*
Mansehra city	4	Mean	13.025	8.3	Present
		Std. Deviation	9.16	6.2	
Dhodial	3	Mean	12	8.8	Present
		Std. Deviation	10.95	7.5	
Shinkiari	5	Mean	2.82	1.32	Present
		Std. Deviation	1.14	0.49	

Table 6. Frequency of *E.coli* Serogroup 0157:H7 by source of well, tube well and hand pump water in the three locations.

---	Positive	Negative	Total	Percent Positive
Wells				
Mansehra City	3	1	4	71.1 %
Shinkiari	6	2	8	
Dhodial	23	10	33	
Sub total	32	13	45	
Tube wells				
Mansehra City	3	1	4	
Dhodial	2	1	3	71.4 %
Sub total	5	2	7	
Hand pumps				
Mansehra City	3	1	4	
Shinkiari	4	1	5	
Dhodial	3	0	3	83.3 %
Sub total	10	2	12	
Grand Total	47	17	64	73.4%

Geometric mean estimates of TPC/ ml from the three sources

The bacterial load measured as geometric mean of TPC/ ml came out to be highest for hand pumps water, followed by tube well water and least for well water. So it revealed that the wells water of district Mansehra was better than the tube wells and hand pumps water as shown in figure 4.

Discussion

Water contamination in term of microbes was observed to be high in three study areas. 73% water samples were positive for *E. coli* (figure no.6).
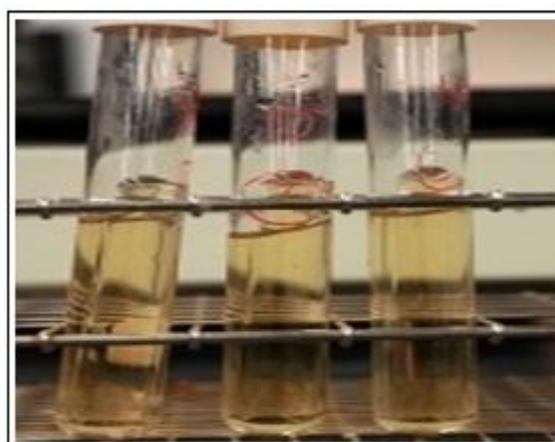

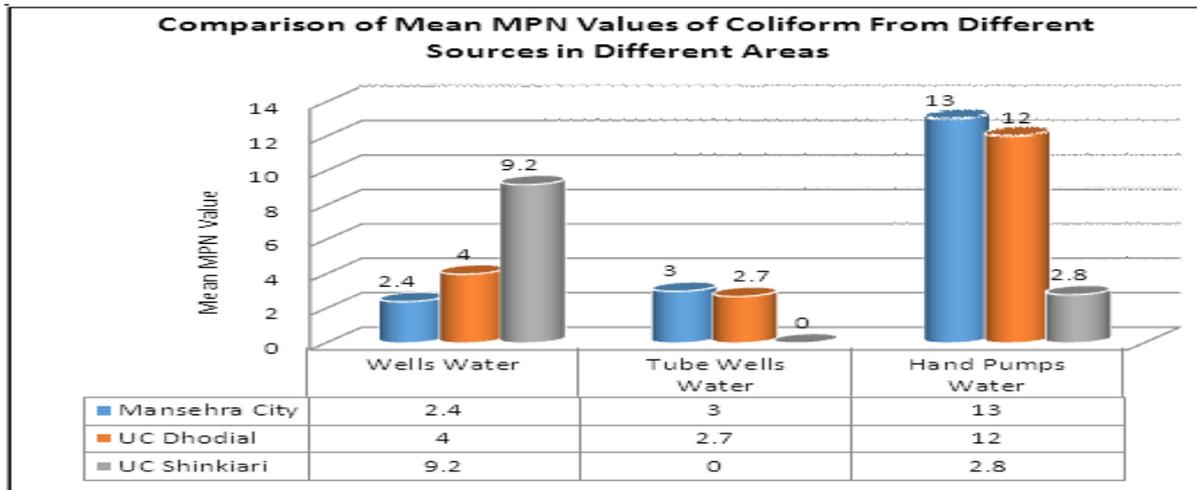**Fig. 1.** Multiple Tube Fermentation method for Coliforms.

Fig. 2. Comparison of mean MPN values of coliform from different sources in different areas.

Figure no. 2 is clearly indicating that coliform contamination was quite high in hand pump water both in mansehra city and union council dhodial as compared to union council shinkiari.
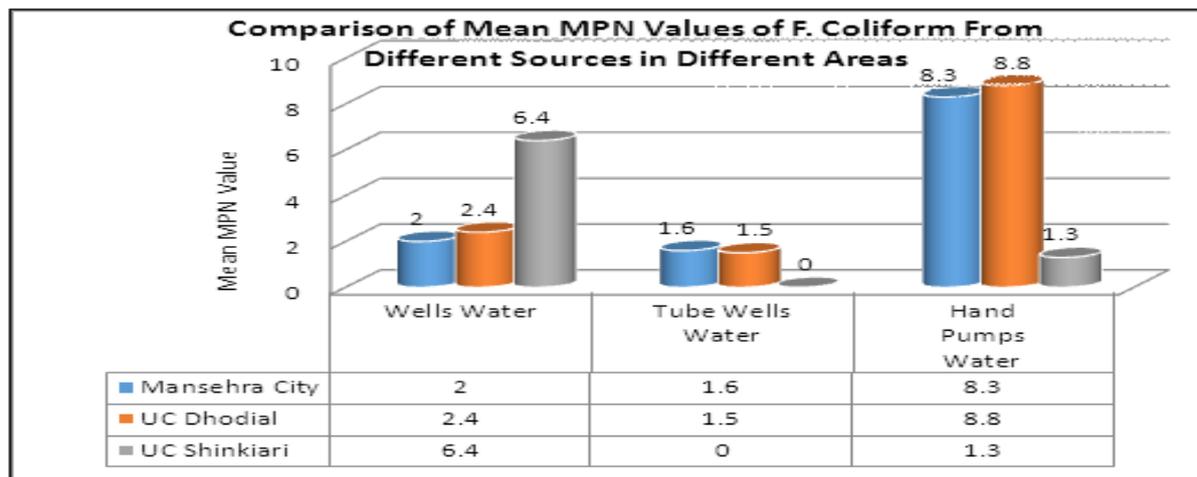

Fig. 3. Comparison of mean MPN values of F. coliform from different sources in different areas.
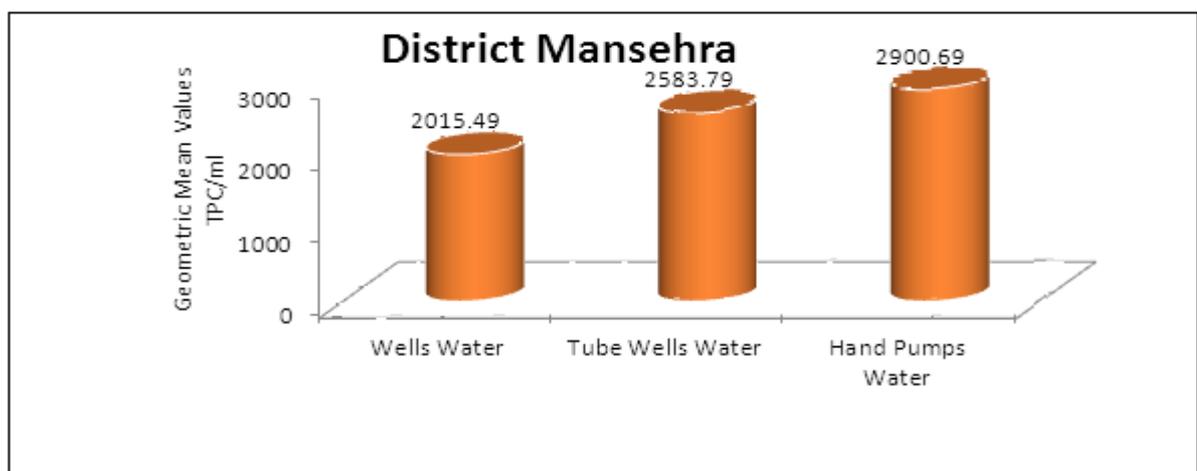

Fig. 4. Comparison of geometric mean values of TPC/ml of three sources from study areas of district Mansehra

Table no. 6 is showing the frequency of *E.coli* in three water sources. 71% samples were positive from wells, 71% from tube wells and 83% from hand pumps.

Well water of shinkiari was more contaminated compared with well water of mansehra city and dhodial. Contamination in term of F.coliform, was high in hand pump water both from mansehra city and dhodial while shinkiari well water was more contaminated. These results are a clear picture of high contamination in hand pump water compared with well and tube well water in mansehra district.

Conclusion

Present study indicates that the only 27% water samples are *E. coli* free, which is very alarming situation and also shows that there is lack of cent per cent purity in any water source from study area i.e. Mansehra district. It recommends some important aspects including; proper sanitary surveys, design and implementation of water and sanitation projects, regular disinfection, maintenance, supervision, and periodic bacteriological assessment of all drinking water sources.

The study was aimed to identify the possible microbial threats to the population by the consumption of contaminated water; similarly it was assessed to find out the spots from where the contamination actually linked. Preventive measures must be taken at all levels to prevent water contamination and the cleanliness of water reservoirs must be mandatory. Drinking water should be boiled before use and protection of water sources should be ensured. In this view low cost measures to be applied at domestic level i.e. chlorination and use of potassium per magnate, the cheapest methods among all.

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