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BACTERIOLOGICAL ANALYSIS OF DRINKING WATER IN URBAN AND RURAL AREAS OF TEHSIL KHANPUR DISTRICT RAHIM YAR KHAN, PUNJAB, PAKISTAN

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Abstract

The availability of safe drinking water is basic need for good health and is also a fundamental human's right.In developing countries like Pakistan,bacterial contamination in drinking water causes severe diseases such as cholera, dysentery, typhoid fever, and many other gastrointestinal infections. Therefore, present study has been designed for bacteriological analysis of drinking water in urban and rural areas of Tehsil Khanpur, District Rahim Yar Khan, Punjab, Pakistan. Water samples were collected from different sources like underground water, water filtration plants, government supply water, and untreated supply water of canals (in villages) using simple random sampling method. Samples were analyzed for bacterial contamination using Total viable count (TVC) and Most probable number (MPN) method. MPN was performed for the detection of coliforms. Spore formers were detected by heat shock treatment,but no sample was found to have spore former. Total 33 out of 105 samples produced colonies on nutrient agar plates. Among 33 positive samples, the highest coliform count was 1600 MPN per 100 in 1 sample. 100-400 coliform count was observed in 4 samples while less than 50 coliform count was examined in 28 samples. The 25 out of 33 samples showed growth on EMB agar confirming presence of Escherichia coli. The results showed that the most of the samples were found contaminated due to uncertain water distribution and improper sanitation. This data may help to develop attentiveness about the need for clean water for policy makers to adopt hygienic drinking water for the community.

Keywords: *Coliform count, Escherichia coli, Most probable number (MPN), Total viable count (TVC)*

INTRODUCTION

The accessibility of clean and safe drinking water is the basic requirement for good health and a fundamental human's right. The impurities in drinking water should be in acceptable amount that it can be safely used for domestic use like irrigation, drinking and cooking. The parameters and guidelines set by WHO (World Health Organization) for water quality have been desecrated continuously with poor handling and checking (1, 2).

Water pollution is common and serious problem of the developing countries. In developing countries, 90 percent of mortality in children under the age of five is due to the contamination of drinking water. In Pakistan, almost 50 percent of diseases and 40 percent casualties happen due to unsafe drinking water reported in community health issues (3). The government of Pakistan conducted an economic survey (2008) which exposed that approximately 50 million populations is rundown of safe and clean drinking water[4]. According to UNICEF (United Nations Children's Fund) statement, around 1.8 million deaths occur annually, and 4 billion cases are due to water contamination[13]. According to WHO, 80% of health problems are due to drinking of contaminated water (1, 4).

Drinking water in Pakistan is obtained from surface or ground water sources. About one third of total water sources of Pakistan is ground water and is a lonely resource of water supplies in main cities (5). Only 36.00% residents of Pakistan have an access to secure and safe water for drinking from which 41.00% of urban population and 32.00% population of rural area is utilizing the safe drinking water in the Pakistan6.

Different areas of Pakistan have contaminated water which carries higher quantities of coli-form bacteria and other disease-causing microorganisms like *Escherichia coli, Vibrio cholera, Salmonella spp., Pseudomonas aeruginosa, Shigella spp.* and *Staphylococcus aureus* which are possible source of waterborne diseases such as dysentery, gastroenteritis, typhoid fever, cholera, diarrhea, and other enteric diseases in children and adults (7, 8).

Khanpur is a Tehsil of district Rahim Yar Khan, Punjab, Pakistan. Water is being contaminated in some areas of Khanpur because there were large ponds that were contaminated by open drains and these ponds have been affecting the underground water quality. However, tests should be performed to assess the quality of water that is being used by the people of Khanpur. The drinking of contaminated water is the main source of transmission of infectious diseases which poses serious threat to the public health. Keeping in view the severity of this problem; present study was conducted for bacteriological examination of drinking water from urban and rural areas of Tehsil Khanpur, District Rahim Yar Khan, Punjab, Pakistan.

METHODOLOGY

STUDY AREA AND SAMPLE COLLECTION

The study was conducted at Department of Microbiology, Government College University Faisalabad, Pakistan. A total of 105 drinking water samples were collected from both urban and rural areas Tehsil Khanpur. The sources of water samples were underground water, water filtration plants, government supply water, and untreated supply water of canals (in villages). After collecting the samples, bottles were labeled with time, date, and location, stored in sunlight-protected boxes and were brought immediately (According to WHO guidelines, the time between sample collection and analysis should, in general, not exceed to 6 hours, and 24 hours is considered the absolute maximum. During that time period, the samples should be transported in lightproof insulated box containing melting ice or ice-packs with water to ensure rapid cooling. If ice is not available, the transportation time must not exceed 2 hours) to the Postgraduate research laboratory, Department of Microbiology, Government College University Faisalabad, Pakistan.

BACTERIOLOGICAL ANALYSIS

The bacteriological contents in drinking water samples of Tehsil Khanpur, District Rahim Yar Khan were analyzed by following techniques:

TOTAL VIABLE COUNT (TVC)

To detect the viable bacterial count, the samples were diluted 10-fold serially and 0.1ml of each water sample was inoculated on nutrient agar plates and incubated at 37 °C for 24-48 hours. The numbers of colonies were counted by using colony counter and Colony forming unit (CFU) was determined by the following formula.

CFU= Numbers of colonies x Dilution Factors/ Sample volume (ml)

IDENTIFICATION OF SPORE FORMING BACTERIA

For the detection of any spore former in water samples, heat shock treatment was applied. For this, water samples were kept on water bath at 90 °C for 15 minutes and immediately cooled at room temperature. After cooling down of water samples, viable count technique was performed as stated above.

COLIFORM DETECTION TESTS

The coliform in water samples were detected by most probable number (MPN) method. The presumptive test was performed by inoculating 10 ml, 1ml and 0.1ml of water samples in three sets of lactose broth tubes (5 lactose broth tubes in each set and immersed inverted Durham's tubes in all sets). Inoculated test tubes were incubated at 37°C for 24 hours. The coliform count was determined using MPN

standard table by Hitchins *et al*., 1998. For the confirmatory test, a loopful culture from each positive fermentation tube was streaked over EMB agar plates. The EMB agar plates were incubated at 37 °C for 24 hours. A concluding test of colonies that appeared after confirmatory test was performed by growth on nutrient agar slants.

RESULTS

TOTAL VIABLE COUNT (TVC)

Among 105 water samples, 33 samples produced colonies on nutrient agar plates. The results of positive samples are given below in Table I (Fig. 1a).

Table I. Shows the number of colonies and colony forming unit (CFU) of each positive sample

Sr. No.	Sample code	Sample location	Source	No. of colonies	CFU
1	U2	Govt. Model High School	Underground water	11	220
2	U3	Model Town A	Water filtration plant	47	940
3	U6	Model Town B	Water filtration plant	86	1720
4	U8	Ghalla Mandi	Government supply water	126	2520
5	U10	Satellite Town	Government supply water	More than 300	
6	U13	Male ward THQ	Underground water	162	3240
7	U14	Emergency THQ	Water filtration plant	13	260
8	U15	Rehman Colony	Government supply water	44	880
9	U16	Gulberg Town	Government supply water	114	2280
10	U20	Akhtarabad	Underground water	58	1160
11	U25	Pakistan Chowk	Government supply water	174	3480
12	U26	Pakistan Chowk	Underground water	156	3120
13	U29	Taraqi Taleem High School	Government supply water	108	2160
14	U45	Akhatarabad phase II	Underground water	84	1680
15	U46	Ranger Public School	Government supply water	90	1800
16	R2	Chak No. 23/P	Government supply water	38	760
17	R4	Chak No. 24/P	Underground water	134	2680
18	R8	Govt. high school Mari Allah Bachaya	Underground water	57	1140
19	R9	Chak 92/1.L	Water filtration plant	84	1680
20	R14	Chak 134/1.L	Water filtration plant	194	3880
21	R15	Chak 134/1.L	Government supply water	62	1240
22	R16	1.L Canal	Canal water	More than 300	
23	R21	Basti Fareed Nagar	Underground water	95	1900
24	R25	Chak No. 27/P	Government supply water	189	3780
25	R27	Bagh-o- Bahar	Government supply water	135	2700
26	R29	Chak No.34/P	Underground water	More than 300	
27	R30	Chak No. 36/P	Government supply water	73	1460
28	R36	Ghulam Nabi Larr	Government supply water	58	1160
29	R38	Basti Abbassia	Underground water	134	2680
30	R44	Basti Khushak Khan	Underground water	98	1960
31	R46	Basti Ameer Buksh	Underground water	72	1440
32	R50	Basti korai	Underground water	45	900
33	R53	Jetha Butha (Hamza Sugar Mills)	Government supply water	288	5760

*All the experiments were performed in triplicates and all results were reproducible. One representative data is depicted in this study (The number of colonies mentioned are the average of triplicates).

IDENTIFICATION OF SPORE FORMING BACTERIA

None of the colony was observed on nutrient agar plate which depicted that water samples were not contaminated with spore forming bacteria.

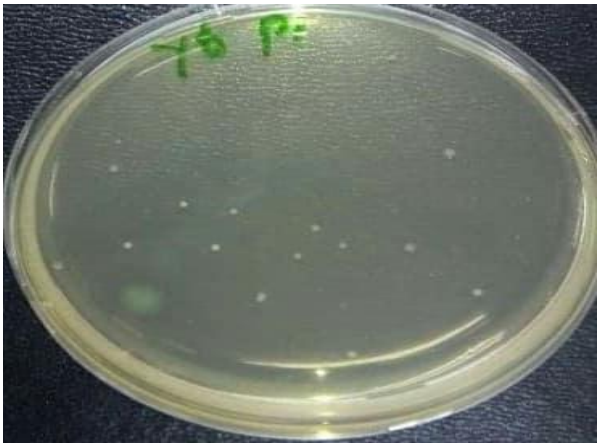
COLIFORM DETECTION TEST

Among 33 positive samples, the highest coliform count was 1600 MPN per 100 in 1 sample. 100-400 coliform count was observed in 4 samples while less than 50 coliform counts was examined in 28 samples. The results are shown in Table II (Fig. 1b and Fig. 2a). Further, on EMB agar plates, 25 samples produced green metallic sheen which confirms the presence of *E. coli* (Fig. 2b).

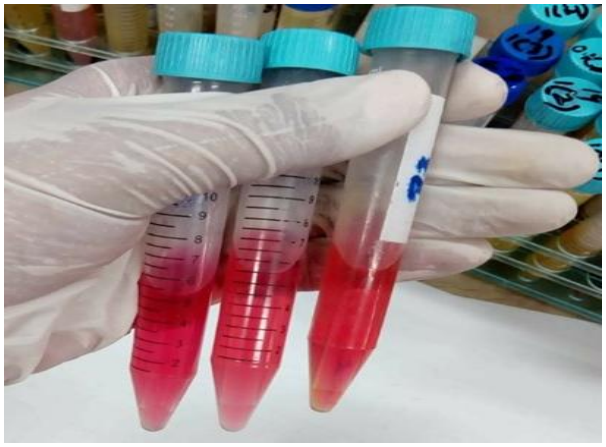
Table II. Shows the results of MPN (5 tubes each at 10, 1, and 0.1 ml) calculated from standard MPN

Sr. No.	Sample code	No. of positive tubes			MPN/100 ml	Confidence limits	
		10ml	1ml	0.1ml		Lower	Upper
1	U2	0	2	1	5.5	1.8	15
2	U3	2	4	0	15	5.9	36
3	U6	1	1	2	8.1	3.4	22
4	U8	3	4	1	24	9.8	70
5	U10	5	4	4	350	100	700
6	U13	2	3	0	12	4.1	26
7	U14	2	1	2	12	4.1	26
8	U15	4	4	2	47	15	120
9	U16	4	3	2	39	14	100
10	U20	1	3	0	8.3	3.4	22
11	U25	0	2	1	5.5	1.8	15
12	U26	2	3	0	12	4.1	26
13	U29	2	2	2	14	5.9	36
14	U45	4	2	3	38	14	100
15	U46	3	2	2	20	6.8	40
16	R2	2	1	0	6.8	1.8	17
17	R4	2	3	1	14	5.9	36
18	R8	3	0	1	11	3.5	23
19	R9	3	4	1	28	9.8	70
20	R13	3	3	2	24	9.8	70
21	R14	4	4	2	47	15	120
22	R15	4	4	1	40	14	100
23	R16	5	5	4	1600	400	4600
24	R25	3	2	0	15	5.7	36
25	R27	0	2	0	3.7	0.70	10
26	R29	5	3	2	140	52	400
27	R30	3	1	2	17	6	36
28	R36	4	2	3	38	14	100
29	R38	2	2	1	12	4.1	26
30	R44	5	3	3	170	70	400
31	R46	3	3	2	24	9.8	70
32	R50	2	3	0	12	4.1	26
33	R53	5	4	3	280	100	710

*All experiments are performed in triplicates and all results were reproducible. One representative data is depicted in this study.



1a



1b

Fig. 1 (a). Showsbacterial colonies on nutrient agar platesof Total viable count (TVC), (b). shows non-lactose fermentation tubes for coliform count by MPN method

2a

2b

Fig. 2 (a). Shows lactose fermentation tubes for coliform count by MPN method, (b). shows greenish metallic sheen produced by *E. coli* isolates

DISCUSSION

The availability of clean and safe drinking water is the essential requirement for good health and is also a primary human's right. The most common health related risk problems are linked with contaminated drinking water. Bacterial contamination causes serious type of diseases such as cholera, typhoid fever, dysentery, and many other gastrointestinal infections. Approximately 75 percent of total world's diseases are water borne (11).

Berihum and Solomon 2018 (12) carried out a research work to identify the coliform in the water that was supplied to consumer for drinking purpose. Samples were processed by applying different methods including bacteriological analysis, and Membrane filtration method (MF). Results showed that unprocessed water samples contained coliform bacteria. Another parallel study was conducted by Khalid *et al*. 2018 (13, 14) assessed the physicochemical and microbial parameters that was related to drinking water pollution of district Vehari. Another study conducted by Ayesha et al. 2020 in different zones of Quetta city on 160 drinking water samples for detection of coliform. Among these 160 samples, eighty nine (55.62 %) were coliform positive while 71 (44.37 %) were coliform negative. Equal number of samples were taken from each zone (40 samples were collected from each zone) It was observed that out of coliform positive; North zone 11.25 %, South zone 11.87 %, East zone 18.75 % and West zone contain 12.50 % contaminated samples with total percentage of 29.37 % found in the domestic water, 18.12 % coliforms found in the restaurants water and 8.12% fecal coliforms have been found in the water of universities, colleges and schools. East zone was highly contaminated with fecal coliform bacteria (15). For this purpose, a total of 41 water samples were obtained from different area of Vehari. 35 water samples out of 41 were obtained from electric pumps and 6 water samples were taken from Tehsil municipal administration of Vehari. Results declared that water samples contained *E. coli* and coliforms which confirmed that biological factors are responsible for causing contamination in the water. In present study, 105 samples were taken for analysis of drinking water. Out of 105 samples, 54 samples were taken from rural areas and 51 samples were taken from urban areas. MPN method was used to assess the presence of coliform bacteria in these samples and 33 samples (35%) were positive. 25 among 33 samples confirmed the presence of *E. coli*. Colonies possessing greenish metallic sheen were observed on EMB agar which is the characteristic feature of *E. coli*. The water samples were found unsafe for people and other domestic purposes.

CONCLUSION

In the present study most of the water samples were found contaminated with coliforms and *E. coli* which indicates the poor quality of drinking water in Tehsil Khanpur. The reason behind this contamination is the mixing of sewage water with drinking water due to poor sewage and sanitary conditions. Further, in Pakistan, most of the water treatment plants are not working properly. In view of this scenario, it is necessary to formulate new guidelines and policies that can help to improve the community health. This

study will help the people to know the importance of safe drinking water. The government should take immediate steps to solve out this issue and provide safe and pure drinking water to consumers.

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