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Bacteriological quality assessment of drinking water from Khyber agency and its impacts on public health

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Abstract: A study was conducted to evaluate the quality of drinking water in Khyber Agency valley, which has always been crucial with reference to public health importance. Quality assessment of drinking water were carried out by determining, total plate count, total coliform bacteria, total fecal coliform, *E. coli* and Staphylococcus aureus. The total plate count (TPC) was found in the range of 3600-190 CFU/ml which indicated that none of the samples were found in drinking water according to the WHO standards (100 CFU/ml). All the samples were found contaminated with total coliform bacteria and unfit for human consumption according to WHO standards. Total fecal coli form bacteria present in 80% samples. *E. coli* were present in 66% samples while 33% analyzed samples were contaminated with Staphylococcus aureus.

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INTRODUCTION

Clean drinking water, a basic necessity is available only to a smaller proportion of Pakistani population. Due to mismanagement, bad civic conditions and natural disasters in developing countries like Pakistan there is a very high ratio of underground water contamination[1]. Water pollution is the specific impairment of water quality by agricultural, domestic or industrial wastes to a degree that has an adverse effect upon any beneficial use of water yet that does not necessarily create an actual hazard to public health. Due to urbanization and industrialization, wastewater that is being discharged into natural water bodies results in serious ground water contamination[2]. The most serious pollutants in terms of human health worldwide are pathogenic organisms. Altogether, at least 25 million deaths each year are blamed on these water-related diseases, including nearly two-third of the moralities of children under five years old. The main source of these pathogens is from untreated or improperly treated human waste [3]. Drinking water supplies have a long history of being infected by a wide spectrum of microbes. Therefore, the primary goal of water quality management from health perspective is to ensure that consumers are not exposed to pathogens that cause disease. Protection of water sources and treatment of water supplies have greatly reduced the incidence of these diseases in developed countries. Therefore, testing the source of water is necessary, especially when there is no water treatment. This is useful as result of the failure of treatment process or as a part of an investigation of serious water-borne disease outbreak[4]. Every person needs approximately 2 liters of clean drinking water and this amount reaches to approximately 12 millions/day for the world population[5].

The poor health status of Pakistan's population is reflected in high infant mortality rate of 12.6 % and as low as 7% fertility rates. The scanty hospital's data shows that many of the diseases treated are caused by water borne microbes indicating that a substantial proportion of morbidity in Pakistan is due to use of polluted water. Gastrointestinal infections resulting in diarrhea show high frequency among children as well as adults, accounting for 25% of patients treated at hospitals and clinics[6,7].

Total coliforms and *Escherichia coli* (*E. coli*), the most common member of fecal coliform bacteria, are the important microbiological parameters of water quality[8]. The presence of *E. coli* in water indicates that the water was contaminated by fecal material of humans or other warm-blooded animals. The U. S. Environmental Protection Agency (EPA) recommended that *E. coli* is a better indicator of fecal pollution than fecal coliform for purposes of evaluating ambient fresh water quality[9]. In this study, the densities of total coliforms and *E. coli* were measured in natural spring waters relating to recreational mountain areas during the summer of 2002 to investigate the presence of coliform bacteria in the drinking spring waters.

MATERIALS AND METHODS

Site description and sample collection

Fifteen samples of drinking water from different sources in Khyber Agency valley were collected and transported by standard methods[10]. Random sampling was adopted for the study. Microbiological analysis of water samples was conducted in Food Microbiology Laboratory, PCSIR Laboratories Complex Jamrud Road Peshawar. All the samples were collected in sterile Duran bottles of 500 ml capacity, properly sealed, kept at 4°C and are analyzed within an hour after procurement.

Microbial analysis

Total plate count

Total plate count was determined by pour plate method, serial dilutions (10^{-1} to 10^{-4}) of the product were made and aliquots of 1ml were added to each duplicate Petri dish. Total Plate Count Agar was added to each Petri dish for total plate count and incubated at 35°C for 48 hours±2, after incubation colony was count by colony counter and result was expressed as CFU/ml[10].

Total coliform bacteria

The MPN of total coliforms bacteria were determined by multiple tube fermentation technique [10]. 1ml from the previously prepared 10^{-1} 10^{-2} and 10^{-3} dilutions were inoculated into three replicate tubes containing 10 ml of LT Broth with inverted Durham tubes and incubated at 35°C±0.5°C for 24 and 48±2 hrs after inoculation. Tubes were examined for evidence of gas production at the end of 24 hrs incubation. Gas production was measured by gas displacement in the inverted vial and also effervescence produced when the tube was gently shaken. Negative tubes were re-incubated for additional 24 hr and again examined for gas production. Positive tubes with gas formation and turbidity were sub-cultured into Brilliant Green Lactose bile broth and incubated at 35°C for 48hrs. Total coliform were calculated from tables as MPN/ ml[10].

Total fecal coliform bacteria

Tubes having 10 ml E.C. broth with inverted Durham tubes was inoculated by means of 3mm loop from the presumptive fermentation tubes showing gas and incubated at 44.5 °C for 24 hrs and examined for gas production. Fecal coliform were calculated from tables MPN/ ml[10].

E. coli

EMB Agar was used for the enumeration of *E. coli*. All the tubes of E.C. broth showing gas were subculture by streaking on EMB agar plates and incubated at 35 °C for 18-24 hrs. Positive plates contained typical colonies with green metallic sheen were inoculated on PCA slants (plate count agar) and incubated at 35 °C for 18-24 hrs. After 24 hrs incubation the typical colonies were confirmed by biochemical tests and also by kits (*E. coli O157:H7* latex test reagent kit Pro Lab. Canada)[10].

RESULTS AND DISCUSSION

The results of bacteriological analysis of drinking water of Khyber Agency were calculated and presented (Table 1). Total plate count (TPC) in sample Jamrud-1 (3600 CFU/ml) was higher among all the analyzed samples, while the lowest was found in Hassan Khail-5 (190 CFU/ml). The TPC were found in Hassan Khail-3 (2800 CFU/ml), Hassan Khail-4 (2200 CFU/ml), Asho Khail-7 (1700 CFU/ml), Asho Khail-6 (1280 CFU/ml), Jamrud-6 (1300 CFU/ml), Jamrud-7 (1080 CFU/ml), Asho Khail-8 (980 CFU/ml), Jamakor-1 (960 CFU/ml), Jamrud-6 (1300 CFU/ml), Jamrud-5 (1160 CFU/ml), Jamrud-4 (990 CFU/ml), Jamrud-3 (692 CFU/ml) and Jamrud-2 (470 CFU/ml). The results of these samples showed that these drinking water samples were not fit for human consumption.

Table 1: Bacteriological analysis of drinking water of Khyber Agency

S#	Sample Location	*TPC CFU/ml	[1]TCB MPN/ml	[2]TFC MPN/ml	[3]*ECi*	[4]*SA*
1	Jamrud-1	3600	>110	25	+ve	-ve
2	Jamrud-2	470	23	12	+ve	-ve
3	Jamrud-3	692	7.5	<0.3	-ve	-ve
4	Jamrud-4	990	4.0	<0.3	-ve	+ve
5	Jamrud-5	1160	110	46	+ve	+ve
6	Jamrud-6	1300	>110	110	+ve	+ve
7	Jamrud-7	1080	>110	24	+ve	-ve
8	Jamakor-1	960	>1100	15	+ve	-ve
9	Jamakor-2	780	46	7.5	+ve	-ve
10	Hassan Khail-3	2800	>1600	12	+ve	+ve
11	Hassan Khail-4	2200	240	4.3	+ve	-ve
12	Hassan Khail- 5	190	Nil	Nil	-ve	+ve
13	Asho Khail- 6	1280	>110	21	+ve	-ve
14	Asho Khail- 7	1700	>110	9.3	-ve	-ve
15	Asho Khail- 8	980	>110	6.4	-ve	-ve

***TPC CFU/ml** = Total plate count colony forming unit/ml
[1]**TCB MPN/ml** = Total Coliform Bacteria Most Probable Number/ml
[2]**TFC MPN/ml** = Total Fecal Coliform Most Probable Number/ml
[3]**EC** = Escherichia Coli
[4]**SA** = Staphylococcus Aurous

Drinking water quality forecast of Peshawar valley on the basis of sample data were studied and it was calculated that water from all sources are according to the WHO recommended standards (Table 2) and fit for supply to consumer. While moving within the distribution system the same water becomes unfit for human consumption due to gradual fall in quality due to mixing of wastewater entering the distribution line through leakage[11].

The study carried out by Najmul H *et al*[12] in Pakistan showed that almost 90% of ground water and well water samples were found to be

contaminated with coli forms and fecal coli forms. Whereas 37% of bottle water samples were showed the presence of coli forms and fecal coli forms. Overall 67% of total water samples were found to be contaminated with coli forms and fecal coliform.

Table 2: Bacteriological standards of drinking water

Bacteriological Parameters	Standards
Total Plate Count	<100cfu/ml
Total Coliform Bacteria	<3 MPN/ml
Total Fecal Coliform Bacteria	<3 MPN/ml
E. coli	- ve
S. *aureus*	Nil

Zubair and Rippy[13] found that high level of contamination of ground water with nitrate and fecal coliform in urban areas of Karachi and suggested that ground water quality was likely affected by waste water infiltration. Khan *et al*[14] find that more than 50 % water samples of Peshawar, Nowshera and Charsada were highly contaminated and were considered unfit for human consumption. Luby *et al*[15,16] used plastic water storage vessel with home chlorination as a potential inexpensive, sustainable means to achieve cleaner water. An inexpensive, home made based water decontamination and storage system was tested in a pilot project in a low income neighborhood of Karachi. The baseline drinking water samples among intervention households were found contaminated with a mean 9397 CFU/100 ml of thermo tolerant coliform compared to a mean 10,990 CFU/100 ml from controls. The results of total coliform bacteria (MPN/ml) indicated that highest TBC>110 (MPN/ml) were observed in Jamrud-1, Jamrud-6, Jamrud-7, Jamakor-1, Hassan Khail-3, Asho Khail-6, Asho Khail-7 and Asho Khail-8. The presence of coliforms in BMW indicates the exposure of water to the external environment, as coliforms are widely spread in the environment.

Hence it can be assumed that the bottled water was exposed at some point of the processing, either before bottling or after. If it happened before processing then chances of source contamination increases such that even the whole water processing of water purification couldn't make it potable and fit for consumers, or otherwise the processing was not up to the standard as required. In such cases the authorities are blamed for not having check and balance on such commercial blunderers. On the other hand the presence of fecal coliforms is of more concern, because it directs the relation of water with fecal contamination. Eighty percent (80%) samples were contaminated with fecal coliform bacteria. Sixty six percent (66%) samples were contaminated with *E. coli*. The bacterial species Escherichia coli is one of the most common inhabitants of the human intestinal tract and is probably the most familiar organism in microbial world. Its presence in water or food is an indication of fecal contamination. It can cause of urinary tract infections, and certain strains produce enterotoxins that cause traveler's diarrhea and occasionally cause very serious food born disease [17]. The presence of fecal coliform and *E. coli* indicate water contamination with human or animal wastes. Diseases causes by microbes (pathogens) in these wastes can cause diarrhea, cramps, nausea, headaches or other symptoms. Theses pathogens may pose a special health risk for infants, young children, and people with severely compromised immune systems. Thirty three percent (33%) samples were contaminated with *S. aureus*. These bacteria produce several toxins that damage tissues or increase the microorganism's virulence. The production of the toxin of serological type A (which is responsible for most cases) is often correlated with the production of an enzyme of that coagulates blood plasma. Such bacteria called coagulase positive. The toxin quickly triggers the brain's vomiting reflex center; abdominal cramps and usually diarrhea then ensue[17].

CONCLUSION

The results of samples are rather alarming and signify that quality of the water is critical in perspective of diseases prevalence and other health problems. Therefore a regular scrutiny of water quality for improvement not only would prevent disease and hazards but also checks the water resources from going further polluted. A variety of factors must be carefully assessed to ensure public health protection. These factors include the treatment processes required to achieve high water quality; the quality of the existing water supply and any changes in this source after recycled water is blended; system reliability; the regulatory framework and risk management practices.

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