Research Article

Biochemical and microbiological evaluation of the water samples collected from different areas of district Kohat and Mohamand Agency, Pakistan.

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ABSTRACT

Contamination of drinking water sources mainly due to microorganisms is the major problem in many areas of Pakistan. Pakistan is also facing the problem of contamination of drinking water which greatly affects human health and quality of life. The most important component of human beings for living is water. Therefore, it is important to analyze drinking water quality mostly in developing countries as the local people are mostly unaware of the water pollution. In this study, twenty three samples of water were analyzed during a 3-month period from the well and lake water supplies of different areas of Kohat and Mohamand Agency. The bacteriological evaluation was done and several tests were performed such as Total Plate Count, *Coliform*, *Faecal coliform*, *Escherichia coli* (*E. coli*) and Biochemical test. In this study, thirteen samples were in the normal range and 10 samples were out of safety ranges fixed by World Health Organization (WHO). The water which was not fit for drinking can be a consistent risk of the infectious diseases and continuous assessment and purification strategies should be developed in these areas to reduce the microbial contamination. The proper training by the local public authorities is required to educate the local community about water pollution, their causes and preventive measures in order to improve the health status of the people in the regions.

Key words *E. coli* and Biochemical test, *Faecal coliform*, *Total coliform*

INTRODUCTION

Water is the most important component for living things and to support the life processes. Around 75% of the human body weight is composed of water. Without this it is not possible to maintain life on this planet. The quantity of water present on earth is approximately 1.4 trillion cubic meters [1-3]. Due to increase in environmental pollution, water resources are contaminated, which is a big hazard to life. This hazard is by regular discharge

of industrial wastes and is a major problem in Pakistan [4]. The main sources of drinking water in Pakistan are tube wells and dug wells. Water gets polluted when its condition or composition or both are changed. The major sources of pollution in natural water are discharge of domestic and industrial wastes from urban, rural and industrial areas. Kohat and Mohamand agency are important areas of Khyber Pukhtoonkhwa (KPK); therefore process of development is also fast. The population

of these areas is constantly increasing. The increase in population give rise to many serious problems, especially increase in environmental pollution. As a result the sources of water are being polluted constantly [5]. Drinking water must be free from turbidity, color and odor as well as from objectionable taste which have different inauspicious physiological effects [6-8].

Fecal coliform bacteria include *Total coliform* and *E. coli* are the important microbiological parameters of water quality. Water contamination indicates the presence of *E. coli* either by fecal material of humans or other warm-blooded animals. According to U. S. Environmental Protection Agency (EPA), *E. coli* is a good indicator of fecal pollution than fecal coliform to evaluate fresh water quality [9-11]. Contaminated water has direct and indirect effect; direct effect is on human health while indirect effect is through consumption of foodstuffs being irrigated with sewage and industrial effluents. World Health Organization estimated that around 80% of the population is deprived of pure drinking water in developing countries and every year around 5 million people die as a result of illness causes due to unsafe drinking water. The major pollutants present in water are mainly heavy metals, microorganisms, fertilizers and other toxic organic compounds [12].

According to World Health Organization estimation every year about 500 million diarrhea cases take place in children usually less than five years in Asia, Africa and Latin America. The diseases like typhoid fever, bacillary dysentery, infectious hepatitis and other enteric infections are commonly transferred through contaminated water. Apart from this many other diseases are also

transmitted through polluted water. Cancer is also caused by the assembly of certain materials carried by water to human organs [13-16]. *Escherichia coli* is a unicellular microorganism, associated with faecal contamination of water. In human faeces, 95% of coliforms are *Escherichia coli* [17-20]. For determination of water contamination and its standards the total aerobic plate count (TAC) is used as an indicator [21]. TAC is a subgroup of *E.coli* used for the indication of faecal pollution of contaminated water [22]. The use of bottled water is being increasing from the past 30 years worldwide [23]. The consumer use bottle water mainly due to safety and potential health benefits because bottled water is much safer than tape water [24, 25]. In Pakistan, rural populations are still deprived of clean drinking water and in urban areas contamination of drinking water is due to leakage in distribution systems [26-30].

The aim of the study was to investigate the microbial contamination in the water samples collected from different areas of Kohat and Mohmand agency. This study will help to specify the areas where water is contaminated and purification strategies should be applied accordingly by the local authorities.

Methods and Materials

Water Sample Collection

Sample collection was performed from February to April (2013) and sampling was done randomly from different areas of Kohat and Mohamand Agency. Around 23 samples were collected from different dug wells, and lakes. The sampling method was according to standard method given by APHA (2005). Samples were collected aseptically in 250 ml sterilized autoclavable glass bottles in the laboratory.

Bacteriological Parameters:

The experiments for bacteriological analysis of drinking water were performed at the laboratories of Pakistan council of scientific and industrial research (PCSIR) complex Peshawar.

Total Plate Count:

Total plate count is determined by standard method. Serial dilution was prepared and aliquots of 1 ml were added to each replica petri dishes. Plate count agar was added to every petri dish for total plate count and incubated at 35°C for 48 hours. After incubation, colonies were formed and then counted by colony counter and results were expressed as CFU/ml.

Total Coliform Bacteria:

Multiple tube fermentation technique was used to determine the total coliforms bacteria. One ml from previously prepared serial dilution was incubated in 3 duplicate tubes containing 10 ml of Lactose Broth with reversed Durham tubes and kept warm at 35°C for 24 and 48 hours. Tubes were observed for confirmation of gas formation at the end of 24 hours incubation. Gas making was calculated by gas dislocation in the reversed vial and bubble formation also occurred when the tubes was shaken smoothly. Unenthusiastic tubes were reincubated for extra 24 hours and observed for a second time for gas making. A positive tube with gas creation and turbidity was subcultured into BGB (Brilliant Green bile broth). All BGB tubes were kept warm at 35°C for 48 hours and checked for gas making. Total Coliform was calculated from MPN table per 100 ml.

Total Fecal Coliform Bacteria

Tubes having 10 ml E.C broth with reversed Durham tubes were kept warm by means of 3 mm loop from the presumptive fermentation tubes

viewing gas and keep warm 44.5°C for 24 hours and observed for gas making.

Isolation of *E.Coli*

EMB Agar was used for determination of *E. coli*. All tubes of *E. coli* broth showing gas were subcultured by streaking on EMB Agar plates and incubated at 35°C for 24 hours. Positive plates contained typical colonies with metallic sheen were inoculated on PCA (plate count agar) slants for morphological and biochemical tests. After 24 hours incubation, the typical colonies were confirmed by biochemical tests and also by kits (*E. coli* O157:H7 latex test reagent kit Pro Lab. Canada) (31).

Results and Discussion

This study was conducted for the identification of bacteria in drinking water samples from different areas of Kohat and Mohamand Agency. In total, 23 samples were collected from the above mentioned areas and checked for the presence of bacterial contamination. The Total Plate Count, Total Coliform, Fecal Coliform, *E. coli* and Biochemical tests were used for the bacterial identification. Seven out of ten samples collected from Kohat were determined to be unfit for drinking due to the presence of *E. coli* as given in Table 1. Three out of 13 samples collected from Mohamand Agency were identified to be unfit for drinking purposes (Table 2). The result revealed that 10 (43.5 %) out of 23 samples have microbial contamination and unfit for drinking. The bacteriological analysis revealed that Total Plate Count (TPC) in the sample collected from Naveed Hotel (867 CFU/ml) was higher among all the samples, while that of Kohat Board (162 CFU/ml) was the lowest. The TPC values of all the samples are given in the mentioned tables where the samples have the TPC

Table 1: The analysis of water samples collected from different localities of Kohat.

S. No	Sample ID	Source	Total Plate Count (CFU /ml)	Total Coliform Bacteria (MPN / 100 ml)	Fecal coliform	<i>E.coli</i>	Date of analysis	Date of report (2013)	Remarks
1	Kohat Bazar	Tape Water	688	9.2	present	present	25-Feb	01-Mar	Unfit
2	Kohat Adda	Tape Water	543	3.6	Present	Absent	25-Feb	01-Mar	Unfit
3	KUST	Tape Water	90	< 1.1	Absent	Absent	25-Feb	27-Feb	Fit
4	Kohat Board	Tape Water	162	3.6	Present	Absent	04-Mar	08-Mar	Unfit
5	B-1 Hostel	Tape Water	73	< 1.1	Absent	Absent	04-Mar	06-Mar	Fit
6	Kakaji Hotel	Tape Water	451	3.6	Present	Absent	18-Mar	22-Mar	Unfit
7	Pak Afghan Hotel	Tape Water	595	9.2	present	present	18-Mar	22-Mar	Unfit
8	Naveed Hotel	Tape Water	867	>23	Present	Present	18-Mar	22-Mar	Unfit
9	Kohat Cantt	Tape Water	67	< 1.1	Absent	Absent	18-Mar	20-Mar	Fit
10	Lake Water	Lake Water	604	>23	Present	Present	18-Mar	22-Mar	Unfit

within safety ranges of WHO was considered as fit for drinking and vice versa. Based on all the data and the given limits of WHO, all the water samples were considered as either fit or unfit for the drinking. The results revealed that the samples collected from lake water were highly contaminated and this could be due to the open access of the lake water to all the available wastes of those areas and no proper sanitation. The lake water contamination is not only alarming for the animals using this water for drinking but also for humans who are using these lakes for swimming in the summer season of Pakistan. In this study, the water belonging to different hotels of the mentioned areas were found highly contaminated and this is a question mark for the local authorities to adopt preventive measures and advise the hotels administration to improve their sanitation and water storage system in order to provide safe and

pure water for drinking and washing. These hotels are mostly located in the surroundings of the Kohat University where mostly the young generation and specifically students are doing their lunch/dinner. This will have long lasting effect on the health of the local community. The spring water was mostly found safe and free of contamination. Mostly these springs are available in the areas have less population and much better environment, however when the spring waters comes in the pipelines and provided to local community, it becomes contaminated. According to previous literature, 90% of rural drinking water supplies system studied were found contaminated with *Coliforms* [31, 32]. The methods used in this study were also previously used to investigate the microbial content of different waters in developing countries. Even some indicators were used to indicate surface contamination of drinking water supplies [33-35].

Table 2: The analysis of the water collected from different areas of Mohmand Agency

S.No	Sample ID	Source	Total Plate Count (CFU /ml)	Total Coliform Bacteria (MPN / 100 ml)	Fecal coliform	E.coli	Date of analysis	Date of report	Remarks
1	Mohamand Bazar	Tape Water	94	< 1.1	Absent	Absent	11-Mar	13-Mar-13	Fit
2	Gandao	Tape Water	488	>23	present	present	11-Mar	15-Mar-13	Unfit
3	Mohamand Edu.academy	Tape Water	89	< 1.1	Absent	Absent	11-Mar	13-Mar-13	Fit
4	Prang Ghar	Tape Water	47	<1.1	Absent	Absent	11-Mar	13-Mar-13	Fit
5	Yaka Ghund Cheena	Spring Water	267	9.2	Present	Absent	15-Mar	19-Mar-13	Unfit
6	Subhan Khawar	Tape Water	91	< 1.1	Absent	Absent	15-Mar	18-Mar-13	Fit
7	Sesai Kelli Khawar	Lake Water	276	9.2	Absent	Absent	23-Apr	26-Apr-13	Unfit
8	Yar Khan Kelli	Tape Water	36	< 1.1	Absent	Absent	23-Apr	25-Apr-13	Fit
9	Khwaga Kelli	Tape Water	64	< 1.1	Absent	Absent	25-Mar	27-Mar-13	Fit
10	Kharef Dara	Tape Water	62	< 1.1	Absent	Absent	15-Apr	18-Apr-13	Fit
11	But Mena Kelli	Tape Water	90	< 1.1	Absent	Absent	22-Apr	24-Apr-13	Fit
12	Pie Khan Kelli	Tape Water	37	< 1.1	Absent	Absent	22-Apr	24-Apr-13	Fit
13	Ambaar	Tape Water	81	<1.1	Absent	Absent	29-Apr	02-Mar-13	Fit

E. coli in drinking water was also used for the identification of recent fecal contamination and for the identification of pathogens, or intestinal parasites [36]. Almost, 15% of the drinking water was reported to be contaminated with *Coliforms* instead of up-to-date standards. In case of major cities such as Karachi (Pakistan) some pathogens such as *Bacillus sp.*, *preudomonads.*, *Flavobacterium sp.*, *Actinomyces.*, and *Micrococcus sp* were reported in water samples where the *Coliform* is absent and this revealed that some pathogens can even inhibit the *Coliform* growth and thus results its exclusion from water [37, 38]. In this study, the water samples collected from the springs or dug wells were found safe, however when these waters moved in the pipelines or stored in the containers, the contamination chances increased. This could be due to lack of

knowledge, leakage of pipelines, improper placement of wells and the poor ways of water supply. Mostly these chances were reported in high ratio during and after rainfall periods [31]. It is very important to educate the local community and hotel administrations regarding the hazards of the water pollution and safety measures to prevent microbial contamination in drinking water.

Conclusion

It can be concluded from the study that the spring and dug well waters were mostly pure and free of contamination, however when these waters go through pipelines the water becomes contaminated. The reason is the unsafe routes and leakage in the pipelines and should be properly managed to reduce contamination. The lake water was highly contaminated as it provides an open space for waste water, industrial solid waste and gutter waste

Table 3: The bacteriological standards fixed by World Health Organization (WHO).

Bacteriological Parameters	Standards
Total Plate Count	<100 cfu/ml
Total Coliform Bacteria	<3 MPN/ml
Total Fecal Coliform Bacteria	<3 MPN/ml
<i>E. coli</i>	- ve

Table 4: Biochemical test for confirmation of *E. coli* in waters of Charsada and Mohamand agency.

S. No	Sample ID	MRVP	Indole test	Latex
1.	Kohat Bazar	Positive	Positive	Positive
2.	Kohat Ada	Positive	Positive	Positive
3.	Kohat Board	Positive	Positive	Positive
4.	Kakaji hotel	Positive	Positive	Positive
5.	Pak Afghan hotel	Positive	Positive	Positive
6.	Naveed hotel	Positive	Positive	Positive
7.	Lake water	Positive	Positive	Positive
8.	Gandao	Positive	Positive	Positive
9.	Yaka Ghund Cheena	Positive	Positive	Positive
10.	Cessai Kelli Khwer	Positive	Positive	Positive

of the local population. The water collected from hotels was also found contaminated and needs proper assessment of the local authorities. Proper training is required for the local population and

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