MedCrave
Step into the World of Research

International Journal of Hydrology

Research Article

Open Access

CrossMark

Drinking water quality, water distribution systems and human health: a microbial evaluation of drinking water sources in salt range

Abstract

In developing countries such as Pakistan, waterborne infections are more common among infectious diseases due to unsafe supply of drinking water and inadequate sanitation systems. In the salt range of Pakistan, sources of drinking water in rural and suburban areas are not protected against microbial contamination resulting from poor sanitation systems and saline water seepage. To assess the bacterial contamination levels and their impact on public health, drinking water samples were collected from various sources in Khewra and Pind Dadan Khan. Total *Coliforms* including faecal *Coliforms, E. coli, Staphylococcus aureus* and faecal *Streptococcus* were determined using the Most Probable Number (MPN) method to quantify the bacterial contamination. The data collected were subjected to statistical analysis to establish the potential relationship between water contamination levels among different sources and health risks posed by different bacterial species. The results revealed the presence of different bacterial species and there was a significant difference in average microbial concentrations among different water supply systems and natural sources. Almost all of the sources were infected either with *Coliform* group, *Staphylococcus aureus* or with faecal *Streptococcus*. An interview-based survey was also conducted to gather information regarding disease incidence rate and results showed a strong relation in the contamination levels and disease prevalence rate.

Keywords: drinking water, human health, microbial assessment, salt range, Pakistan

Volume 2 Issue 5 - 2018

Mateen Shafqat,[1] Aniqa Batool,[1] Syeda Sabahat Kazmi,[2] Saima Imad,[2] Muhammad Asad Ghufran,[3] Nafeesa Samad[1]

[1]Department of Environmental Sciences, Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi, Pakistan

[2]National Physical and Standardization Laboratories, Islamabad, Pakistan

[3]Department of Environmental Sciences, International Islamic University Islamabad, Pakistan

Correspondence: Aniqa Batool, Lecturer, Department of Environmental Sciences, Pir Mehr Ali Shah-Arid Agriculture University Rawalpindi, Shamsabad Murree Road Rawalpindi, Pakistan 46300, Tel +92-322-5374712, Email aniqabatool@uaar.edu.pk

Received: January 30, 2018 | **Published:** September 10, 2018

Introduction

In developing countries, most of the population is exposed to a wide range of enteric pathogens and suffers numerous episodes of gastrointestinal illnesses as a result. Microbiologically contaminated drinking water, unsafe supplies of water for personal hygiene and underprivileged sanitation, are the main contributors to an estimated 4 billion cases of diarrhea each year causing 2.2 million deaths, mostly among infants.[1] Waterborne infectious diseases are caused by certain types of microbes present in drinking water, which is related to inadequate water supply, and/or contamination of water sources. The most familiar pathogens found in water are total *coliforms*, *E. coli, Staphylococcus, Salmonella spp., Shigella spp., Cryptosporidium*, *Legionella spp*., *Vibrio cholerae* and some viruses like Hepatitis virus.[2] By 2001, a total of 1415 species of infectious microbes known to be pathogenic to humans had been identified.[3] Diseases like diarrhea, cholera, typhoid and sometimes hepatitis are also referred with water as a medium to cause and spread of such diseases because of their presence in water. Poor sanitation system is considered as a major cause of such diseases.[4] The extent of bacterial contamination in drinking water is strongly related to seasonal trends as well as geographical variations.[5] The capacity of bacteria to survive and grow at alkaline pH values is of widespread importance in the epidemiology of pathogenic bacteria present in saline waters either used for drinking or any other purpose.[6] *E. coli* when grown in a buffer medium at pH 7.0, *E. coli* cells exhibit a generation time of 18 minutes, whereas at a pH level of 8.7 the cells exhibit a generation time of 25 minutes.[7]

The drinking water supply sector has mostly targeted the waterborne spread of pathogens. The most common method in use is the chlorination of drinking water at treatment plants and in the supply systems. Chlorination is considered vital to make it safe for drinking, especially when water is obtained from surface water sources. Although there are worldwide acceptance and use of drinking water chlorination in epidemic outbreak situations, there is an ongoing discussion on whether the interruption of waterborne transmission of pathogens reduces the occurrence of endemic diarrheal diseases in rural areas in developing countries.[8–10] However, in the case of Pakistan, most water-supply systems are not working according to design, and many are completely dysfunctional.[11] This raises the question whether improvements in these schemes and provision of microbiologically safe drinking-water would have an impact on the incidence of diarrheal disease.[11] The available water in the salt deposited areas contains high amounts of sodium, chlorides, nitrates, magnesium and fluoride etc.[12] In the salt range of Pakistan, drinking water resources are not protected from chemical and microbial contamination resulting from poor sanitation systems and rainwater runoff. Health complaints by residents of different locations of the salt range including hypertension, stone formation in kidneys, dental fluorosis, limb deformities, skin problems, nausea and abdominal diseases are very common. The microbial contamination of drinking water is also very common due to leakage in pipelines and cross connections with draining lines and also due to unsafe water storage practices. In the current perspective, it is presumed that use of contaminated water for drinking purposes pose varying degree of health problems to the local residents. The objectives of this study were to generate a comprehensive baseline data on the microbial status of drinking water from different residential areas and to identify the strength of association between water contamination level and overall disease incidence and morbidity rate in salt range.

Material and methods

Major localities

The salt range is a strip of hill system in Punjab Province deriving its name from extensive deposits of rock salt. The range extends

from Jhelum River to Indus across the northern portion of Punjab Province. The most populated areas in the salt concentrated strip are Khewra, Pind Dadan Khan and Choa Saiden Shah (Figure 1). Khewra is a crowded town located close to Khewra Salt Mines and Pind Dadan Khan is an administrative subdivision of Jhelum District with a settlement of over 0.7 million people. The area considered as the primary target was Khewra and Pind Dadan Khan because of the maximum concentration of salt in drinking water owing to salt mining activities in Khewra and rainwater runoff from mines towards Pind Dadan Khan.

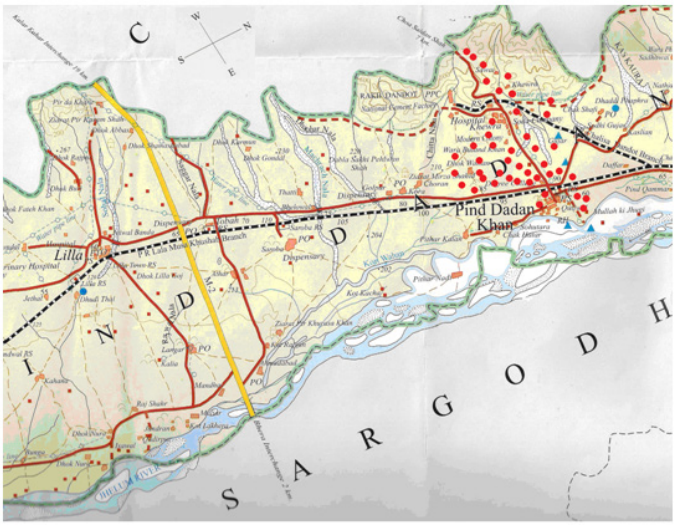

Figure 1 Red dots (•) representing sampling sites in Khewra and Pind Dadan Khan (courtesy Google maps).

Major water sources

Different water supply agencies are supplying water to the population living in areas of Khewra and Pind Dadan Khan. The main sources of water on which these agencies depend are Jhelum River, ICI (Imperial Chemical Industries) outlets, boreholes and natural springs. In premises of Pind Dadan Khan, almost 90% of the total population is dependent upon TMA (Tehsil Municipal Administration) water distribution system for drinking and other domestic purposes.

Sampling and transportation

A total of 51 water samples were collected from different water sources in Khewra and Pind Dadan Khan. Pre-sterilized glass bottles were used as sample containers. A solution of 0.3% sodium thiosulphate was added to store the samples in a ratio of 0.1ml in 120ml of the sample; a temperature of 4°C was maintained during transportation, and the samples were subjected to microbial analysis within 6 hours after collection of samples.[13,14] All the samples were collected according to the protocol set by FAO (Food and Agriculture Organization) and SMWW (Standard Methods for the Examination of Water and Wastewater).[13,14]

Analytical procedures

Different indicator species including total *coliforms,* faecal *coliforms* and *E. coli*, *Staphylococcus aureus* and faecal *Streptococcus* were assessed in drinking water samples by the Most Probable Number (MPN) method. This method was performed using the Multiple Tube Fermentation Technique (MTFT). Water samples were inoculated into sterile media in glass tubes selected for the determination of specific indicator species of microorganisms. All the methods followed for the analysis of different types of bacteria were according to SMWW and FAO.[13,14]

Survey of disease incident rate

A survey was conducted to collect monthly data from hospitals, dispensaries and local clinics regarding the prevalence of different waterborne diseases for duration of one year. In this study, the waterborne diseases were focused in order to understand the relationship between the contamination levels of water and the rate of disease occurrence related to that specific disease.

Statistical analysis

ANOVA was performed to obtain data to investigate the difference between contamination levels of various types of water sources. Disease incidence rate was also assessed to understand its relationship with the level of microbial contamination, and the vulnerability of people exposed to saline water from a health perspective in salt the range of Pakistan.

Results and discussion

The average value for Most Probable Number Index per 100ml (MPN/100ml) of total *Coliforms* group was 54.88 MPN/100ml which was relatively higher than *Staphylococcus aureus* and faecal *Streptococcus* having average counts of 38.25 MPN/100ml and 10.68 MPN/100ml respectively (Figure 2). Faecal *Streptococcus* was observed with the lowest average MPN Index among all other microbial parameters. Microbial counts in different water sources (TMA Supply, ICI outlets and Natural Springs) were also determined which were found to be different in concentrations from each other. Analysis of drinking water quality samples clearly indicated that the contamination level of water was increasing at an alarming rate that may pose a serious threat to human health and the environment. Microbial contamination of drinking water supplies, as well as other natural sources, may be either due to the failure of the disinfection of the raw water at the distribution systems or to the infiltration of infected water (sewage) through cross connections or leakage points. In piped supplies, the likelihood of microbial contamination increases with discontinuity of supply as well as the increased pressure of the surrounding medium i.e. soil or water which may contain infected effluent from leaked sewers.[15] Municipal sewage contains human faeces and water contaminated with these effluents may contain disease-causing organisms and consequently, may be harmful to human wellbeing if used as drinking water or in food preparation.[16]

Comparative significance of microbial counts in drinking water samples

ANOVA was performed to compare the average MPN counts among three microbial parameters (total *Coliforms*, *Staphylococcus aureus* and faecal *Streptococcus*) which clearly indicated a significant difference between all parameters (Figure 2). The average microbial counts of total *Coliforms* were found to be significantly higher ($p = 0.0001$) than faecal *Streptococcus*. Similarly, average counts of *Staphylococcus aureus* in water samples were found significantly higher ($p = 0.0059$) than faecal *Streptococcus*. Although there was no significant difference observed among microbial counts of total *Coliforms* group and *Staphylococcus aureus*, but both species were found abundant in samples analyzed. Total *coliforms* group was found to be present in 71% of the total water samples. However,

Citation: Shafqat M, Batool A, Kazmi SS, et al. Drinking water quality, water distribution systems and human health: a microbial evaluation of drinking water sources in salt range. *Int J Hydro.* 2018;2(5):542–547. DOI: 10.15406/ijh.2018.02.00123

Staphylococcus aureus and faecal *Streptococcus* were not very much common in samples (55% and 28% respectively). Statistical analysis has shown significant difference among average microbial counts of different microbial species. The microbial standards by WHO suggest a complete absence of total *coliforms*, *Staphylococcus aureus* and faecal *Streptococcus* from drinking water. The maximum microbial counts in drinking water samples were of total *coliforms*. The contamination of drinking water with total *coliforms*, E. coli and faecal *coliforms* is suggestive to much more related with the incidence of diarrhea in children under the age of 5 years and adults.[17]

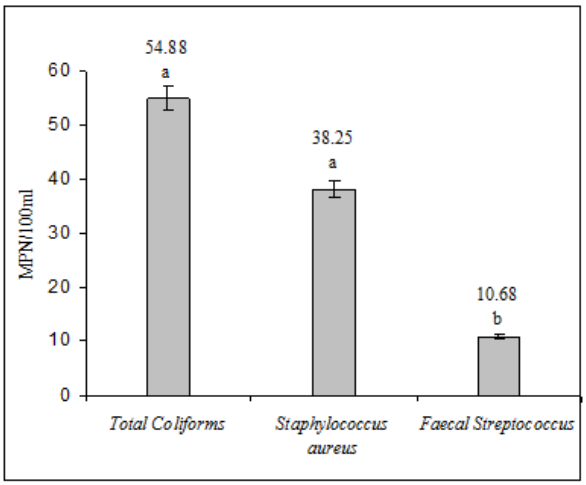

Figure 2 Average microbial counts in water samples taken from different sources.

Microbial counts in different types of water sources

All the water sources were categorized to obtain an overview on contamination intensity in terms of microbial counts. Analyses results of water samples from hospitals, public water taps, schools, households, natural water springs and ICI plant water outlets were combined to form a comprehensive data list indicating contamination level (Table 1). The difference in average microbial counts found in local water supply systems prominently indicated their quality. Among different water supply networks, the TMA supply was found to be responsible for posing serious threats to human health and the environment. The maximum average counts of faecal *Coliforms* were found in samples taken from TMA supply channels or houses. The TMA network for supplying drinking water was found to be highly contaminated with total *Coliforms* group, *Staphylococcus aureus* as well as faecal *Streptococcus* as compared to natural springs and ICI water outlets.

Hospitals: The drinking water samples from hospitals were found free from total *Coliforms* contamination. However, contamination with *Staphylococcus aureus* was at its peak in water from hospitals (average counts=93.5MPN/100ml). On the other hand, only one water sample from THQ Hospital was found contaminated with faecal *Streptococcus* having a concentration of 1 MPN/100ml (Table 1). Water samples taken from hospitals found free from total *Coliforms*. This is probably because of installation of water filters equipped with UV radiations that are capable enough to disinfect a few types of bacteria. However, microbial counts of *Staphylococcus aureus* and faecal *Streptococcus* were determined in samples taken from hospitals probably due to development of resistance against various water treatment technologies.[18]

Table 1 Average microbial counts in water samples taken from different sources

Sources	*No. of Samples*	Total coliforms	*Staphylococcus aureus*	*Faecal streptococcus*	WHO standard
		---------- *Average. Counts (MPN/100ml)* --------			*MPN/100ml*
Hospitals	4	0	93.5	0.25	0
TMA Public Taps	5	96.2	43.8	0	0
ICI Public Taps	5	7.6	9.6	0	0
Natural Springs	6	106.83	8	5	0
Households	22	65.9	39.5	23.36	0
Schools	5	3.16	57.5	0.16	0
Hotels & Restaurants	3	57.67	16	0	0

TMA public water taps: A total of 5 water samples taken from public taps at different points in the city, which clearly indicated the presence of total *Coliforms* and *Staphylococcus aureus* with average counts of 96.2 MPN/100ml and 43.8 MPN/100ml respectively. Faecal *Streptococcus* were not detected in samples taken from public taps built by TMA (Table 1).

ICI water outlets: Water samples from outlets of ICI Chemical Plant were contaminated with average counts of 7.6 MPN/100ml and 9.6 MPN/100ml for total *Coliforms* including E. coli and faecal Coliform; and *Staphylococcus aureus*, respectively. Faecal *Streptococcus* was not detected in water samples taken from ICI outlets posing this supply as a better option (Table 1).

Natural springs: Samples from the natural water springs present in the outskirts of Khewra town were also highly contaminated with total *Coliforms* (106.83 MPN/100ml). The average microbial counts for *Staphylococcus aureus* and faecal *Streptococcus* were 8 MPN/100ml and 6 MPN/100ml, respectively (Table 1). Maximum microbial counts of *Coliforms* were determined in water samples taken from natural springs present in the surroundings of Khewra town. The water from Mitha Patan spring is being supplied to two-third population of Khewra town by PMDC Water Supply System (Pakistan Mineral Development Corporation). The microbial analyses of water samples taken from these natural springs were not satisfactory because all the samples were contaminated with high concentrations of *Staphylococcus aureus* and faecal *Streptococcus* as well. Water for drinking purposes from Mitha Patan spring is supplied by PMDC to all facilities within the premises of Khewra Salt Mines.

Growth and high counts of Coliform bacteria depend upon high temperature and rainwater runoff which carries animal and human wastes in major water reservoirs used as sources of drinking water for many communities.[19] The study also revealed that the presence of faecal Coliform showed the contamination of water with animal and human faecal wastes, while the total Coliform counts indicate the presence of bacteria from other resources like soil.

Households: A total of 22 water samples were taken from different houses from Khewra and Pind Dadan Khan City. Analysis results showed average counts of 65.9 MPN/100ml, 39.5 MPN/100ml and 23.36 MPN/100ml for total *Coliforms*, *Staphylococcus aureus* and faecal *Streptococcus*, respectively. Figure 3 clearly indicates the contamination level of total *Coliforms* in different households.

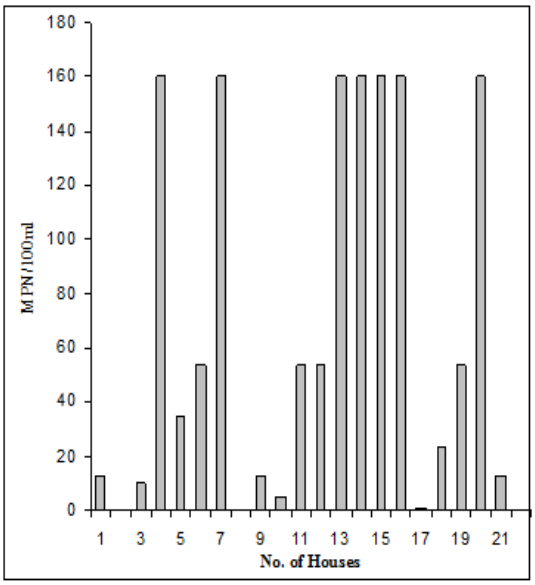

Figure 3 MPN Index of total *Coliforms* in samples taken from households.

Household water samples clearly represent the level of contamination which is responsible for causing waterborne diseases in the study area. Lack of literacy and hygienic practices, housewives are also responsible for the occurrence of diseases like diarrhea etc. in children under age of 5 years as well as adults.[20]

Schools: The highest average microbial counts calculated in water samples taken from schools was of *Staphylococcus aureus* (57.5 MPN/100ml). The average microbial counts for total *Coliforms* and faecal *Streptococcus* were 3.16 MPN/100ml and 0.16 MPN/100ml, respectively (Table 1). Samples taken from several educational institutes from Khewra and Pind Dadan Khan were also not safe from contamination with *Coliforms* and *Staphylococcus aureus*. Faecal *Streptococcus* was also determined from these samples but with very lower counts. Hotels and restaurants: Samples taken from different hotels and restaurants were also free from faecal *Streptococcus* contamination. But a higher microbial count was determined for the presence of *Coliforms* and *Staphylococcus aureus*. The microbial counts of total *Coliforms* and *Staphylococcus aureus* in water samples taken from hotels and restaurants were found to be 57.67 MPN/100ml and 16 MPN/100ml respectively (Table 1).

The significance of microbial counts in different water supply systems

ANOVA was performed to assess the significance of microbial concentrations in different water supply systems and resources. Three major water sources (TMA Supply, ICI outlets and natural springs) were subjected to statistical analysis i.e. TMA supply, natural water springs and private boreholes. According to the data obtained, there was no significant difference between the three water resources (Natural Springs, TMA supply, ICI outlets & Boreholes) regarding contamination of drinking water with total *Coliforms* group (Figure 4). The p-value for analysis between springs and TMA supply was 0.32, between TMA Supply and Boreholes was 0.17, and between springs and Boreholes was 0.3 (Figure 4). However, a significant difference was observed between springs and TMA supply regarding microbial counts of *Staphylococcus aureus* (p=0.038). The counts of *Staphylococcus aureus* were also found significantly different in TMA supply and boreholes (p=0.0074). However, no significant difference was observed in the mean counts of springs and boreholes (p=0.3089) (Figure 5). On the other hand, there was no significant difference observed among counts of faecal *Streptococcus* in samples from springs and TMA supply line (p=0.055). However, there was a significant difference among the samples from TMA supply and private boreholes (p= 0.03). No such difference was found in the microbial concentrations in samples of springs and boreholes (p=0.059) (Figure 6).

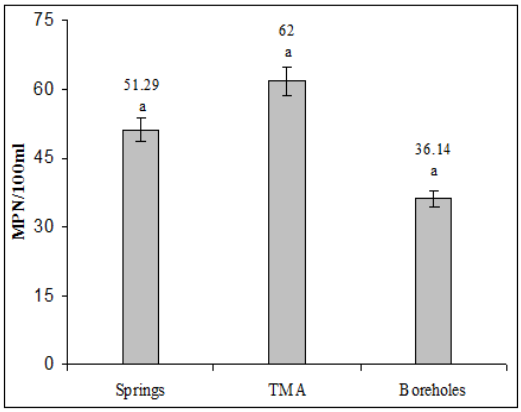

Figure 4 Average counts of total *Coliforms* in different water sources.

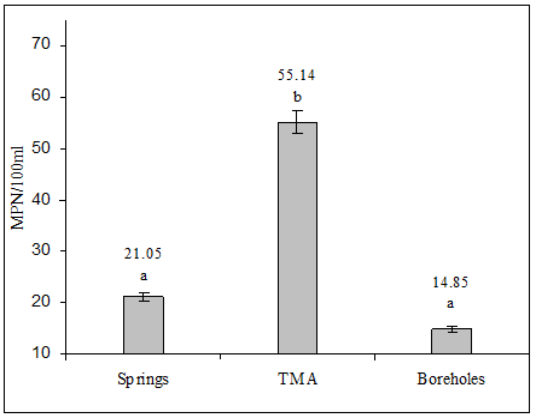

Figure 5 Average counts of *Staphylococcus aureus* in different water sources.

Citation: Shafqat M, Batool A, Kazmi SS, et al. Drinking water quality, water distribution systems and human health: a microbial evaluation of drinking water sources in salt range. *Int J Hydro.* 2018;2(5):542–547. DOI: 10.15406/ijh.2018.02.00123

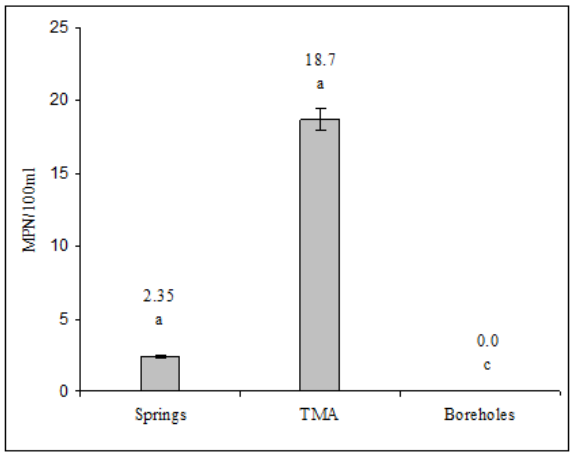

Figure 6 Average counts of faecal *Streptococcus* in different water sources.

According to WHO guidelines[17] all the positive water samples with microbial counts more than 1MPN/100ml exceeded the permissible limits for drinking water. Water used for drinking purposes must be free from the presence of any kind of bacteria including indicator species. Considering microbial standards devised by WHO, no *Coliforms* should be present in drinking water, however, contamination of water samples with *Coliforms* should not exceed 5% of 40 samples collected for monthly assessment of water quality.[21] Similarly, *Staphylococcus aureus* and faecal *Streptococcus* are also set compulsory not be present in drinking water as they can cause skin diseases and abdominal disorders in humans.[21] Intestinal *enterococci* are typically excreted in the faeces of humans and other warm-blooded animals. Intestinal *enterococci* are present in large numbers in sewage and water environments polluted with sewage or wastes from humans and animals (Junko, 2001). Presence of any member of this group indicates the water as not suitable for drinking purposes.[17] In this study, it became clear that the situation of drinking water quality from a microbial perspective in the Salt Range of Pakistan is not merely close to the requirements devised by WHO.

Survey of disease incidence rate in Khewra and Pind Dadan Khan

The survey results of disease incidence rate in the population living in premises of Khewra and Pind Dadan Khan indicated that contaminated drinking water is a serious threat to public health in the region (Table 2). In Tehsil Headquarters Hospital (THQ) Pind Dadan Khan, visits for diarrheal patients under the age of 5 years were recorded as an average of 152 patients per month and 108 patients per month above the age of 5 years. The greater number of diarrheal patients visits in hospital proves the strong relationship between poor water quality and disease incidence rate in developing countries.[20] In THQ Hospital, a daily report is prepared for the prevalence of gastroenteritis, according to which about 25% of outdoor patients belong to diarrhea, and child mortality rate is increasing due to gastrointestinal diseases. According to data obtained by interviewing physicians practicing in Khewra and Pind Dadan Khan, about 80 % of diseases prevailing in the Salt Range are waterborne. Skin diseases become more common in winter due to the presence of *Staphylococcus aureus* in water. Females and children are more vulnerable to waterborne diseases because of unhygienic practices, poor economic conditions and lack of awareness.

Table 2 Monthly data of patients visits to hospitals and clinics in Khewra and Pind Dadan Khan for the duration of one year

No. of cases reported at hospitals in Khewra and Pind Dadan Khan during july-09 to june-2010												
Diseases	Jul 15	Aug 15	Sep 15	Oct 15	Nov 15	Dec 15	Jan 16	Feb 16	Mar 16	Apr 16	May 16	Jun 16
Acute Respiratory Infections	322	344	452	785	791	1230	752	877	744	323	327	642
Pneumonia <5yrs	8	15	12	7	3	13	25	37	25	12	6	5
Pneumonia >5yrs	4	12	4	1	1	4	8	22	20	6	2	10
TB Suspects	24	22	20	23	47	110	6	16	31	4	3	23
Chronic Obstructive Pulmonary Disease	0	0	0	0	0	0	0	78	2	0	0	0
Asthma	43	82	64	57	76	80	87	267	108	11	40	110
Diahrrea <5 yrs	181	85	71	53	40	59	175	41	151	337	474	156
Diahrrea >5 yrs	143	121	48	12	41	30	65	45	94	176	242	282
Urinary Tract Infections	136	151	158	189	142	187	204	185	165	113	164	168
Suspected Malaria	170	165	128	78	81	90	14	65	38	147	33	71
Suspected Meningitis	0	10	0	0	0	1	2	0	0	3	38	1
Suspected Measles	0	0	0	0	0	0	15	0	0	8	0	1
Suspected Viral Hepatitis	32	6	4	0	0	5	0	20	30	3	30	5
Hypertension	4	0	28	65	78	80	91	123	113	87	47	68
Skin Diseases/Skin Allergy	102	131	143	156	137	130	142	61	134	76	7	87
Depression	37	1	5	0	7	10	3	62	15	12	18	22

Conclusions and suggestions

In the salt range of Pakistan, drinking water resources in the rural areas are not protected from chemical and microbial contamination resulting from poor sanitation systems and rainwater runoff. The microbial contamination of drinking water is also very common due to leaking pipelines and cross connections with draining lines. The outcome of the study showed that almost all of the water resources were contaminated either with *Coliform* group, *Staphylococcus aureus* or with faecal *Streptococcus*. It is concluded from the study that the availability and supply of safe drinking water in Salt Range of Pakistan was inadequate. People were using contaminated water provided either by different authorities or from general water sources available. Lack of awareness was also a vital factor that was playing an important role in unhygienic conditions to promote the incidence of waterborne diseases in the study area. However, health status can be improved by some initiatives that could be taken at the administrative level. Following are some recommendations that can be helpful to improve health status in the region:

a. Creating awareness about hygiene and sanitation practices, incidence and transmission of waterborne diseases.

b. Installation of drinking water treatment plants to improve the quality of drinking water in the research area.

c. Installation of pipelines to transport water from springs/rivers to decrease the threat of faecal contamination of water.

d. Up gradation of water supplying infrastructure in order to maintain a safe infrastructure for safe water distribution.

e. Creation of better economic conditions can also help the residents to resist against common waterborne diseases.

Acknowledgements

We are very thankful to whole staff of National Physical and Standardization Laboratories (NPSL), Islamabad. Especially Saima Imad, for her support and assistance in analytical work.

Conflict of interest

The author declares there is no conflict of interest.

References

1. WHO. Global Water Supply and Sanitation Assessment Report. *World Health Organization*, Geneva. 2000.
2. Strauba TM, Chandler. Towards a unified system for detecting waterborne pathogens. *Journal of Microbiological Methods.* 2003;53(2):185–197.
3. WHO. *Emerging Issues in Water and Infectious Disease*. World Health Organization, Geneva. 2003.
4. Fewtrell L, Kaufmann RB, Kay D. Water, sanitation, and hygiene interventions to reduce diarrhea in less developed countries: a systematic review and meta-analysis. *Lancet Infectious Diseases*. 2005;5(1):42–52.
5. Ashbolt NJ. Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*. 2004;198(1-3):229–238.
6. Padan E, Bibi E, Ito M, et al. Alkaline pH homeostasis in bacteria: New insights. *Biochimica et Biophysica Acta*. 2005;1717(2):67–88.
7. Maurer LM, Yohannes E, Bondurant SS, et al. pH regulates genes for flagellar motility, catabolism, and oxidative stress in *Escherichia coli* K-12. *Journal of Bacteriology.* 2005;187(1):304–319.
8. Esrey SA. Water, waste, and well-being: a multicountry study. *American Journal of Epidemiology*. 1996;143(6):608–623.
9. Cairncross S, Kolsky P. Water, waste, and wellbeing: a multi country study. *American Journal of Epidemiology*. 1997;146:359–360.
10. VanDerslice J, Briscoe J. Environmental interventions in developing countries: interactions and their implications. *American Journal of Epidemiology*. 1995;141(2):135–144.
11. Jensen PK, Jeroen HJ, Ensink, et al. Effect of Chlorination of Drinking-water on Water Quality and Childhood Diarrhoea in a Village in Pakistan. *Journal of Health Population and Nutrition.* 2003;21(1):26–31.
12. McCarthy MF. Should we restrict chloride rather than sodium?. *Medical Hypotheses*. 2004;63:138–148.
13. APHA. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, Washington DC 2005.
14. FAO. Manual of Food Quality Control. 4 Rev 1. Microbial analysis. FAO Food and nutrition paper. *Food and Agriculture Organization of the United Nations. Rome.* 1992.
15. Prasai T, Lekhak B, Joshi, et al. Microbiological analysis of drinking water of Kathmandu valley. *Scientific World*. 2007;5(5):112–114.
16. Bartram J, Balance R. Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. *In: Microbiological Analyses*. UNEP/WHO 1996.
17. WHO. *Water sanitation and hygiene links to health*. WHO. 2004.
18. Cebrián G, Sagarzazu N, Pagán R. Development of stress resistance in *Staphylococcus aureus* after exposure to sublethal environmental conditions. *International Journal of Food Microbiology*. 2010;140(1):26–33.
19. Zamxaka M, Pironcheva G, Muyima NYO. Microbial and physicochemical assessment of quality of domestic water sources in selected rural communities of the Eastern Cape Provine, South Africa. *Water SA*. 2004;30:333–340.
20. Grace E, El-Azar, Habib RR, et al. Effect of Women's Perceptions and Household Practices on Children's Waterborne Illness in a Low Income Community. *Eco Health*. 2009;6(2):169–179.
21. WHO. *Guidelines for drinking-water quality*. World Health Organization, Geneva. 2011.