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Flood disaster in Charasadda, Pakistan: Bacteriological examination of drinking water

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Abstract

The current study was conducted to investigate the drinking water quality of flood affected areas of district, Charasadda. A total of 156 samples were collected; 73 house-wells, 35 motor pump and 48 hand pump in sterile 100 ml of flask, representing 20 affected villages. Samples were serially diluted into 10 ml, 1ml and 0.1 ml. Most probable number (MPN) technique was used for the counting of total coliform, fecal coliform and Escherichia coli. The results showed an exceeding value of MPN index / ml and range of 95% probability in House Wells (≥ 60 MPN), Hand Pump (> 41MPN) and Motor Pump (>20 MPN). The biochemical investigations showed that *E.coli* contributed 62%, *Salmonella* 21% and *Shigella* were 16% in total samples. Presence of large counts of bacteria bacillary dysentery, Typhoid fever and Para typhoid fever were common among flood affecters.

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Introduction

Clean drinking water has become the primary concern of people living in developing countries (Joyce *et al.,* 1996). During flooding events ground water contaminated with biological waste, herbicide residues, sewage and other contaminants (CDC, 2010). The monitoring of the microbiological growth in drinking water mainly depends on bacterial indicators such as coliform, fecal coliform, *Escherichia coli* and other gram negative organism (Zmirou *et al.,* 1987, 1995). The fecal coliform test is a primary indicator for the "potablity" of water (Anderson, 2003). Water pollution causes number of diseases like diarrhea, salmonolosis, typhoid, Para typhoid (Atlas and Bertha, 1997). Diarrhea and dysentery are major causes of morbidity (WHO, 2004). Worldwide more than 1 billion people have no access to drinking water sources (WHO, 2004-6). *E.coli, Klebsiella, pneumonia, Shigella dysenteriae, Proteus vulgaris* and *Salmonella typhi* were found in a recent study in Lagos, south western Nigeria, in wells used as drinking water sources (Akinyemi *et al.,* 2006). Cholera were responsible for more than 16,000 diarrheal cases in the West Bengal flood disaster in 1998 and partly responsible (apart from enterotoxigenic *Escherichia* coli) for more than 17,000 cases in the Bangladesh flood disaster in 2004 (Sur, 2000; Qari *et al.,* 2005). Flooding was significantly linked to paratyphoid fever (caused by *Salmonella enteric* Para typhi A) in Indonesia between 1992 until 1993 (Vollaard *et al.,* 2004). In Mozambique in 2000, diarrhea was one of the prominent illnesses observed during the post-flood period (Kondo *et al.,* 2002). When the Hurricane Katrina hit the United States (US) in 2005, diarrheal illness was reported among the evacuees and later found to be caused by nor *virus, Salmonella* and *V. cholera* (CDC, 2005-6). During the Johore flood disaster, a total of 1,996 AGE (acute gastro enteritis) cases, 46 food poisoning cases and no cholera or typhoid cases were detected at all. Only four cases detected in 2000 whereas the typhoid cases were mainly detected among the immigrants (Jabatan & laporan, 2006-7). World Health Organization estimated that up to 80% diseases in the world are caused by polluted water (WHO, 1997).

Drinking water contaminated with pollutant contributes to a heavy burden of waterborne disease in developing countries (Pruss *et al.,* 2002; Moszynski, 2006). Microbial waterborne diseases also affect developed countries (Medema et al., 2003). The assessment of drinking in wells is essential due to main sources of water for human consumption (NPC, 2009). Drinking water Parameters fall under three categories namely microbiological testing, physical and chemical testing (Scott *et al.,* 2003). Waterborne diseases cause 1.8 million deaths each year while about 1.1 billion people have no access to safe drinking water (Clansen *et al.,* 2007). World Health Organization estimated that up to 80% of all sicknesses and diseases in the world are caused by inadequate sanitation, polluted water or unavailability of water (WHO, 1997). The purpose of this study to monitor drinking water contamination of the flood affected area, risk factor for the water contamination and diseases spreaded due to contaminated water.

Materials and methods

Study area

Charasadda District in the Khyber PukhtunKhwa Province, Pakistan. It is located at 34°8'43N and 71°43'51E with an altitude of 276 meters (908 feet) and lies 29 kilometers from the provincial capital of Khyber PukhtunKhwa (KP), Pakistan.

Sampling

A total of 156 samples were collected from twenty villages. The sample were collected in 100 ml sterile conical flasks (Pyrex) from each source and stored at 0-10°c in ice box. The samples were brought to department of Microbiology, Hazara University for further biological analysis.

Statistical technique

For statistical analysis results were obtained with MPN index / ml and range of 95% probability.

Enumeration of bacteria

Samples were serially diluted into 10 ml, 1.0 ml and 0.1 ml respectively. Most probable number (MPN) was used for total coliform and fecal coliform including E.coli. Total coliform was measured in sample by presumptive test, using Lactose broth. Lactose Broth (L.B) broth were prepared under sterile condition and poured into sterile test tube, before pouring small Durham tubes were inserted in test tubes. Test tubes were incubated at 37 °c for 24 hour. Growth was found in tubes after 24 hour. To confirm total coliform presence, growth inoculums was shifted to EMB (Eosin Methylene Blue) media by wire loop for total coliform in tubes. Complete test were perform for fecal coliform using again Lactose Broth. Before pouring broth in tubes Durham tubes were inserted, tubes were incubated at 44c for 24 hour. Gas production after 24 hour in tubes conform the presence of fecal coliform in water. For the presence of other bacteria in water, samples were streaked on specific growth media.

Biochemical test

Biochemical test were performed for identification of different gram negative, enteric organism found in water. Four different type of medias; Simon citrate (SC), Triple sugar iron (TSI), Tryptone water (TW) and Methyl red vogues proskauer (MR-VP) were used. Slant and Butt were made of Simon citrate and triple sugar iron (TSI) in tubes. Inoculation of unknown organism occurs by help of wire loop. MR-VP and Tryptone broth were prepared, inoculation of organism occurs. All tubes were incubated at 37 ◦c for 24 hour. After 24 hour kovak reagent are mixed in Tryptone water while in MR and VP treated with different reagent. MR and VP were treated with Methyl red and Barrett's reagent respectively. On the basis of different color and gas production organism are identified as a different species (Table 2).

Results and discussion

In natural disasters flood were highly destructive. During flooded situation human community, Plants habitats, Soil and water reservoir are heavily affected. In current investigation the ground water reservoir were studied for fecal contamination. The topographical study shows that the area were found slope in nature. The slope nature of area provides rapid flow of water, reducing number of coliform those villages found on top location. In NWFP (Currently known Khyber PukhtunKhwa), the floods velocity increases due to flashy hill and steep slopes (Jawad, 2009). During last week of July, 2010 flood hits the district Charasadda of Khyber PukhtunKhwa, Pakistan. Under certain conditions, water sources severely affected by fecal coliform from a variety of sources (Casteel *et al.,* 2006).To study landscape of area, samples were collected in a descending order from different villages. The descending order was helpful to measure the level of fecal contamination in villages. The main source of water in this area are House well, Hand pump and Motor pump. Samples were collected from these sources. Total coliform, *Escherichia coli* and *enterococci* were used as indicators for water contamination the reason were found that this organism have no reliability to survive without a suitable host (Toranzos *et al.,* 2001).

Water act is a basic unit for maintenance of all forms of life (Pat, 1992; Kegley and Andrews, 1998). Monitoring of drinking water necessary for public health maintenance (NRC, 2004; Savichtcheva and Okabe, 2006).

MPN index/ ml and range of 95% probability shows that total water sample collected from different villages sources; 73 from house well, 48 from hand pump and 35 from motor pump were not potable (Table 1). All villages sample shows grater MPN index/ ml and range of 95% probability which grater then WHO standard. Biochemical test shows different characteristics of *E.coli, salmonella* and *Shigella* (Table 3). In total sample *E.coli* were found 62%, *Shigella* 16% and *salmonella* 21% (Table 3).Two standard methods accepted by the (EU, 2006) for the enumeration of indicator microorganisms in waters. The multiple tube fermentation technique, which provides a Most probable number (MPN) of microorganisms after

cell growth in broth, and the Membrane filtration (MF) technique, which enumerates the colonies grown on the surface of an adequate solid media providing a colony-forming unit (CFU) count (Standard Methods, 1998). MPN method was performs in case of highly contamination; Membrane filter technique becomes fail due to clogging of filter use for water analysis (Geissler *et al.,* 2000; Toranzos *et al.,* 2007).

Table 1. Results of given test showing gas production in Lactose broth, sample reading, MPN index and range of probability for each water sample.

			Gas production												
			Lactose Broth 10 ml Tubes			Lactose Broth 1 ml Tubes			Lactose Broth 0.1 ml Tubes			Sample Reading	MPN INDEX per (ml)	Range 95% probability	
S.No	Village	Water sample	1	2	3	4	5	6	7	8	9			Low	High
1	**Suleykamar**	Mp	-	-	-	-	-	-	-	-	-	0-0-0	<0.03	0.00	0.095
		Hp	-	-	-	-	-	-	-	-	+	0-0-1	0.030	0.0015	0.096
		Hw	-	-	-	+	+	+	-	-	-	0-1-0	0.030	0.012	0.18
2	**Razar**	Mp	-	-	-	-	-	+	-	-	+	0-1-1	0.061	0.012	0.18
		Hp	-	-	-	-	+	+	-	-	-	0-2-0	0.062	0.012	0.18
		Hw	-	-	-	+	+	+	-	-	-	0-3-0	0.094	0.036	0.38
3	**Dubandi**	Mp	-	-	-	-	-	-	-	-	+	0-0-1	0.030	0.0015	0.11
		Hp	-	-	-	+	+	+	-	-	-	0-1-0	0.030	0.013	0.18
		Hw	-	-	-	-	-	+	-	-	+	0-1-1	0.061	0.012	0.18
4	**Bar Bahram Dheri**	Mp	-	-	-	-	+	+	-	-	-	0-2-0	0.062	0.012	0.18
		Hp	-	-	-	+	+	+	-	-	-	0-3-0	0.094	0.036	0.38
		Hw	-	-	-	-	-	+	-	-	+	0-1-1	0.061	0.012	0.18
5	**Zyam killi**	Mp	+	-	-	-	-	-	-	-	-	1-0-0	0.036	0.0017	0.18
		Hp	+	-	-	-	-	-	-	-	+	1-0-1	0.072	0.013	0.18
		Hw	+	-	-	-	-	-	-	+	+	1-0-2	0.11	0.036	0.38
6	**Bahram Dheri**	Mp	+	-	-	+	-	-	-	-	-	1-1-0	0.074	0.013	0.20
		Hp	+	-	-	+	-	-	+	-	-	1-1-1	0.11	0.036	0.38
		Hw	+	-	-	+	+	-	-	-	-	1-2-0	0.11	0.036	0.42
7	**Sher pao**	Mp	+	-	-	+	+	-	+	-	-	1-2-1	0.15	0.045	0.42
		Hp	+	-	-	+	+	+	-	-	-	1-3-0	0.16	0.045	0.42
		Hw	+	+	-	-	-	-	-	-	-	2-0-0	0.092	0.014	0.38
8	**Gander bala**	Mp	+	+	-	-	-	-	-	-	+	2-0-1	0.14	0.036	0.42
		Hp	+	+	-	-	-	-	-	+	+	2-0-2	0.02	0.045	0.42
		Hw	+	+	-	-	+	-	-	-	-	2-1-0	0.15	0.037	0.42
9	**Showdag**	Mp	+	+	-	+	-	-	+	-	-	2-1-1	0.20	0.045	0.42
		Hp	+	+	-	-	-	+	+	+	-	2-1-2	0.27	0.087	0.94
		Hw	+	+	-	+	+	-	-	-	-	2-2-0	0.21	0.045	0.42
10	**Quaid Abad**	Mp	+	+	-	+	+	-	+	-	-	2-2-1	0.28	0.087	0.94
		Hp	+	+	-	+	+	-	+	+	-	2-2-2	0.35	0.087	0.94
		Hw	+	+	-	+	+	+	-	-	-	2-3-0	0.29	0.087	0.94
11	**Sardaryab**	Mp	+	+	-	+	+	+	-	-	+	2-3-1	0.36	0.087	0.94
		Hp	+	+	-	+	+	+	-	-	+	2-3-1	0.36	0.087	0.94
		Hw	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
12	**Abazey**	Mp	+	+	+	-	-	-	-	-	+	3-0-1	0.38	0.087	1.1
		Hp	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
		Hw	+	+	+	-	-	-	-	-	+	3-0-1	0.38	0.087	1.1
13	**Bar Banday**	Mp	+	+	-	+	+	+	-	-	+	2-3-1	0.36	0.087	0.94
		Hp	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
		Hw	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
14	**Hisara Nehri**	Mp	+	+	-	+	+	+	-	-	+	2-3-1	0.36	0.087	0.94

		Hp	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
		Hw	+	+	+	-	-	-	-	-	+	3-0-1	0.38	0.087	1.1
15	**Ginday**	Mp	+	+	+	-	-	+	-	-	-	3-1-0	0.43	0.09	1.8
		Hp	+	+	+	-	-	-	-	-	+	3-0-1	0.38	0.087	1.1
		Hw	+	+	+	-	-	+	-	-	-	3-1-0	0.43	0.09	1.8
16	**Hisara Barani**	Mp	+	+	+	-	-	-	-	-	-	3-0-0	0.23	0.046	0.94
		Hp	+	+	+	-	-	-	-	-	+	3-0-1	0.38	0.087	1.1
		Hw	+	+	+	-	-	-	-	+	+	3-0-2	0.64	0.17	1.8
17	**Station Killi**	Mp	+	+	+	-	-	+	-	-	-	3-1-0	0.43	0.09	1.8
		Hp	+	+	+	-	-	+	-	-	+	3-1-1	0.75	0.17	2.0
		Hw	+	+	+	-	-	+	-	+	+	3-1-2	1.2	0.37	4.2
18	**Dosehra**	Mp	+	+	+	-	-	+	+	+	+	3-1-3	1.6	0.40	4.2
		Hp	+	+	+	-	+	+	-	-	-	3-2-0	0.93	0.18	4.2
		Hw	+	+	+	-	+	+	+	-	-	3-2-1	1.5	0.37	4.2
19	**Daki**	Mp	+	+	+	-	+	+	-	+	+	3-2-2	2.1	0.40	4.3
		Hp	+	+	+	-	+	+	+	+	+	3-2-3	2.9	0.90	10.
		Hw	+	+	+	+	+	+	-	-	-	3-3-0	2.4	0.42	10.
20	**Amer Abad**	Mp	+	+	+	+	+	+	-	-	+	3-3-1	4.6	0.90	20.
		Hp	+	+	+	+	+	+	-	+	+	3-3-2	11.	1.8	41.
		Hw	+	+	+	+	+	+	+	+	+	3-3-3	>11	4.2	>60

Table 2. Biochemical test for identification for different organism identification.

Organism	**Gram staning**	**Methyl red**	**Vogosposkar**	**Citrate**	**Catalase**	**Oxidase**	**Triple sugar iron**				**MIU medium**		
							Slop	**Butt**	**H2S**	**GAS**	**Mot**	**Indole**	**Urea**
E.coli	Rod, -	+	-	-	+	-	Y6	Y	-	+2	+5	+2	-
shiggela	Rod, -	+	-	-	+	-	R	Y	-	-3	-	d	-
Salmonella Typhi	Rod, -	+	-	-	+	-	R	Y	+ weak	-	+	-	-

Key: VP=voges-proskauer, H2S=hydrensulphide gas (blackening), d=different strains give different results, R=red pink (alkaline reaction), Y=Yellow (acid reaction),-= negative, += positive.

The physical appearance of the water was analyzed before water collection. Water color was found light greenish yellow. For coliform measurement presumptive test, complete test and conformed test were performed (Prescott *et al.,* 2005). The presumptive test enlists all organism of family enteriobactericeae high number in water sample. The total coliform was further conformed by conformed test with help of EMB Media (Eosin Methylene Blue). As discuss above E.coli as an indicator for fecal contamination compared to other family member of coliform group. To measure

specifically *E.coli* in water positive samples were taken from EMB Media (Eosin Methylene Blue) and shifted to Lactose Broth (L.B broth). It was found that E.coli was present in water sample.

Table.1 shows that MPN index / ml and Range of 95% probability were increased as measured number of coliform from village to village. Compare MPN index / ml and Range of 95% probability of two villages shows that number of coliform were increased as move from top to bottom with respect to villages location. Through biological analysis it was cleared that number of coliform was found high in case of House well, moderate in case of hand pump and very less in case of motor pump showing variability due to open surface, less depth and long stay of flood water in wells.

Table 3. Total numbers of samples and their sources.

Genus	Total sample	% of total sample	Source					
			Motor pump	sample %	Hand pump	sample %	Well water	sample %
E.coli	97	62%	23	23%	31	31%	43	44%
shiggela	25	16%	5	20%	8	32%	12	48%
Salmonella typhi	34	21%	7	20%	9	26%	18	52%
	Total-156		Total-35		Total-48		Total-73	

Study examined that water contamination were found in all water samples, for pathogenic bacteria water samples were further analyzed. Water sample were passed with the comprehensive analysis of Gram staining, Catalase test, Oxidase test and other biochemical test including TSI and MIU medium test.

Through Gram staining it was clear that all organisms were found gram negative, bacilli rods. Morphological identification was very helpful for organism identification (Braun, 1946 Finkelstein and Punyashthiti, 1967; Lankford and Burrows, 1965).

Triple sugar iron (TSI) test differentiate different organism of family Enteriobactericeae due to glucose fermentation, acid production from other intestinal bacilli. TSI test divided the organism into three genera; *E.coli, shigella* and *salmonella*. TSI (triple sugar iron) for *E.coli,* found High yellow (slope), yellow (Butt), High gas production but no H_2S gas. *Shigella* was found red pink (Slope), yellow (Butt) and no production of H_2S and glucose fermentation. *Salmonella* were found with weak production of H_2S gas. Motility, Indole and urea (MIU medium) were analyzed for *E.coli, Shigella* and *salmonella* typhi. SIM agar medium showed high Mortality for *E.coli*, normal for salmonella and no mortality for *shigella*. Indole was found positive for E.coli, variable for *Shigella* and no Indole activity were found for *salmonella typhi*. Urea test shows that no degradable activity of Urease enzyme for organisms. MR-VP medium activity shows that all organisms were found MR (methyl red) positive and VP (vogues proskauer) negative when treated with Methyl red and barrettes reagents respectively. Citrate utilization ability for all organisms was found negative. Catalase enzyme was found in their genome with positive Catalase test. The cytochrome activity was analyzed with Oxidase test found negative for organism.

Around the world different flood disaster were recorded with different mortality and morbidity. In 1992-3 in Indonesia the out beaks of Para typhoid fever (caused by *salmonella enteric* Para typhi A) were recorded due to contamination of water (Vollaard et al., 2004). Current study also shows fecal coliform and *salmonella typhi* infection. In

1998 more than 16,000 diarrheal out breaks were recorded in Bangladesh flood disaster while in 2004; 17,000 cases were recorded of diarrheal infection (Sur 2000; Qari *et al.,* 2005). Diarrheal diseases were found a major outbreak in Mozambique flood disaster in 2000 was because of contaminated water (Kondo *et al.,* 2002). In current study diarrheal cases were also found in flood affected area, Charasadda.

In 2000 in Johore Malaysia *vibrio cholera* were found in flooded areas. The epidemiological study shows that no cases of vibrio cholera were found before flood disaster. A few cases were examined in foreigners who were come to Johore state. In current study no case of vibrio cholera were found in this area. *Nor virus, salmonella* and *vibrio cholera* were found in 2005 when USA Katrina struck by flood. In current study no case of *Nor* was virus found. See around the globe water contamination is the problem for under developed countries and also for developed countries. Developed counties identified quickly source of contamination due to which risk of disease were decrease.

USA epidemiological shows that, each year 560,000 people suffer severely, 7.1 million suffer mild to moderate with waterborne diseases and total 12,000 deaths were recorded (Medema *et al.,* 2003). Worldwide, approximately 1.1 billion people have no access to safe water and 1.7 million people were died every year from waterborne diseases (Cutter & Miller, 2005). According to WHO report 3 million death were occur due to contaminated water in developing countries (Anon, 997).

Conclusion

Bacteriological analysis shows that the area were contaminated because of flood water due to which it was recommended that a quick response were necessary to wash out the Wells, Hand pump and Motor pump with the help of big motors to reduce the rate of contamination. Secondly add chlorine gas, ozone gas with the contaminated water for purification. On urgent basis send medical teams to affected area to provide medical treatment to the patient affected by different diseases. Create awareness against the different diseases emerged by contaminated water with the help of NGOs worker.

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