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Original Research Article

Drinking Water Analysis of Danish Abad (Peshawar) for Pathogenic Bacteria (*Solmonella, Shigella* and *Escherichia coli*)

Shahid Iqbal[1]*, Muhammad Mehran Anjum[2], Nawab Ali[2], Mujaddad ur Rahman[1] and Azam Hayat[1]

[1]*Department of Pharmacy, Abbottabad University of Science and Technology-Pakistan*
[2]*Department of Agronomy, The University of Agriculture Peshawar-Pakistan*

Corresponding author.

Abstract

Water is the major constituent of all living things and needed by them for various purposes. Drinking water expose human to a variety of contaminants in which most common is microbial contamination. The present study was designed to document the prevalence of pathogenic bacteria including *Shigella, Salmonella* and *Escherichia coli*. A total of 100 water samples were collected and analyzed for bacterial count. These samples were collected from different sources in 20 water distribution systems. The TPC was determined by culturing water samples on plate count agar with pour plate method. SS agar was used for the identification and conformation of *Salmonella* and *Shigella* species of bacteria, while *E. coli* was confirmed by biochemical tests. Out of total, 70% samples were above the WHO guideline value at source point and 94.3% at point of use. The mean value of TPC was higher 4.33×10^{3}cfu/ml at point of use as compare to 2.16×10^{1} cfu/ml at source point. The prevalence rate of *Shigella, E. coli* and *Salmonella* were 32%, 10% and 8% respectively. The study concluded that most of the water samples were unfit for drinking because it have high TPC value and not meet to WHO standards. The water was more contaminated at point of use as compare to source point. Therefore high health risks are associated with consumption of this water.

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Introduction

Water is the major constituent of all living things and needed by them for various purposes. The demand for quality drinking water had changed considerably with the development. In olden days, the only requirement of drinking water was that it should be free flowing and non turbid (Pavendan et al., 2011).

Drinking water expose human to a variety of contaminants, which may be microbial, chemical or other solid waste material. The sources of contamination of drinking water can be divided in two categories: Contaminants in ground and surface water and Contaminants used or formed during the treatment and distribution of drinking water. Contaminants in ground and surface water occur due to natural substances leaching from soil, run-off from agricultural activities, controlled discharge from sewage treatment works and industrial plants and uncontrolled discharges or leakage from landfill sites and from chemical accidents or disasters. In drinking water many chemicals and

microbes founds which are called pollutants of drinking water. Commonly bacteria, fungi, yeasts, protozoa etc are founds as microbiological contaminants in drinking water (Yildirim et al., 2010).

In developing countries everyone has no access to safe drinking water so there waterborne infections are more common. According to the WHO, the mortality of water associated diseases exceeds 5 million people per year. Most of them are microbial intestinal infections, and cholera is most common of them. The great risk is involved in drinking that water which is contaminated with human or animal feces. Wastewater discharges in fresh waters are the major source of fecal microorganisms, including pathogens. In developing countries one of most important public health problem is microbial diarrheal diseases. Mostly those people are affected by diarrheal diseases having lowest financial resources and poorest hygienic facilities. In Asian and African countries children under five are most affected by microbial diseases which are transmitted through water. Waterborne diseases not only affect the people in developing countries but also in developed countries. It has been estimated that each year 560,000 people suffer from severe waterborne diseases, in USA (Cabral, 2010).

In developing nations there is high prevalence of diseases such as diarrhoea, typhoid fever, cholera and bacillary dysentery which is due to the consumption of unsafe water and unhygienic drinking water production practices. The water pollution becomes more dangerous when faecal contaminants enter the water supply. Some pathogens like *Salmonella* species, *Shigella* species, *Vibrio cholerae* and *E. coli* shed in human and animal feces and within treated waste water enter to ground. The *E. coli* are used as indicators of possible sewage contamination because *E. coli* are most commonly present in human and animal feces (Oyedeji et al., 2010).

According to World Health Organization (WHO) there are 4 billion cases of diarrhoea and 2.2 million deaths annually; one of the major causes of this disease is the use of unsafe water. In major causes of water pollution one of cause is increase in human populations and urbanization. The water which is physically colorless, odorless and even tasteless is not sufficient to determine that the water is safe for drinking. The drinking water should be examined microbiologically and physicochemically (Chan et al., 2007).

In large cities of Pakistan diarrhea is leading cause of death in children, it is estimated that 200,000 deaths per year occur due to diarrheal diseases. These diarrheal diseases occur as a result of lack of safe drinking water. The mortality rate of children under five years of age in Pakistan is 101 deaths per 1,000 children, with much of these deaths attributable to water borne diarrheal infections (U.S. Agency for International Development / Pakistan, 2008)

The microorganisms which cause waterborne diseases includes genus *Salmonella* which are gram-negative, these are motile while straight rods in shape. According to biochemical tests they are oxidase-negative and catalase-positive, they produce gas from D-glucose and use citrate as carbon source. The genus *Salmonella* has two species *Salmonella enterica* and *Salmonella bongori*. The *Salmonella enteric* have further sub species and each sub species had different serovars. In humans beings *Salmonella* cause two types of salmonellosis. (1) Typhoid and paratyphoid fever, (2) Gastroenteritis. Infective doses of *Salmonella* is low (less than 1,000 cells) but these are sufficient to cause clinical symptoms. *Salmonella* gastroenteritis is caused by *Salmonella* serovars such as *typhimurium*. Drinking contaminated water after 12 hrs symptoms appears and last 2–5 days. The symptoms are diarrhea, vomiting and fever. The humans and animals intestinal tract is habitat of *Salmonella*. It also found in environment because they are excreted by humans and animals. The sources of contamination in natural waters are sewage, agriculture pollution, and storm water runoff. In the natural environment *Salmonella* don't multiply significantly but it can survive several weeks in water (Cabral, 2010).

The genus *Shigella* is also a Gram-negative, non-spore forming, non-motile bacteria. *Shigella* is straight rod-like in shape. They ferment sugars but there is no gas production. *Shigella* is biochemically oxidase-negative and catalase-positive. The genus *Shigella* has four species. *Shigella dysenteriae*, *Shigella flexneri* and *Shigella sonnei*. For *Shigella* the incubation period is 1–4 days. The disease begins with symptoms like fever, anorexia, fatigue and malaise. Stools are bloody in small volume and a long with abdominal cramps. After 12 to 36 hrs diarrhea progresses to dysentery, blood, mucus and pus appearing in feces. In the initial step of pathogenesis *Shigella* penetrate the colonic mucosa. *Shigella* infection is characterized by degeneration of the epithelium and by an acute

inflammatory colitis in the lamina propria, which cause ulceration of the mucosa and leakage of blood and mucus occur into the intestinal lumen. *Shigella* enters to epithelial cell by adhere to its target cell. *Shigella* produces two types of toxins. Cytotoxic shiga toxin and endotoxin. *S. dysenteriae* serotype 1 produce high level of shiga toxin while *S. sonnei* and *S. flexneri* produce in lower amounts. Shiga toxin inhibits mammalian protein synthesis and lipopolysaccharide (LPS) endotoxin causes an inflammatory response. *Shigella* is typically found in the intestinal tract of humans and other primates. They enter to water by fecal-contamination and then contaminate drinking water and food (Cabral, 2010).

E. coli strains found also in water are involved in intestinal diseases. The pathogenic *E. coli* strains causes' infantile gastroenteritis. After ingestion of contaminated water, watery diarrhea occurs lasting for several days and may leads to dehydration. It is more common in children below 5 years of age. It is also common in developing countries but not common in developed countries. The pathogenic *E. coli* transmits by water contaminated with human feces from an ill individual (Cabral, 2010).

Keeping in view the the importance of water quality on human health the present study was conducted to determine the prevalence of *Shigella, Salmonella* and *E. coli* in drinking water, to know about the sources of contamination in drinking water and to aware the people of the area about the drinking water contamination.

Materials and methods

Study area

Danish Abad a town of Peshawar was selected for the present study. Most people of the area depend upon the tube wells for the drinking water while some have their own bore wells. The area have a total 20 water tube wells, 16 of which have storage tanks while the rest have direct supply to houses. Out of 20 tube wells 4 have open pipe systems (on surface of ground) while remaining 16 were under ground. A total of 100 water samples were collected in sterile plastic containers under strict aseptic condition to reduce chances of environmental contamination. These Samples were collected from all type of water supply, source point and point of use. After collection these samples were transported within an hour to the Microbiology Laboratories of Abasyn University Peshawar for further processing.

Microbial analysis of samples

For microbial analysis these samples were cultured with pour plate method on plate count agar. Before culturing the samples plats were labeled properly. Three different quantities of sample (0.01, 0.1 and 1 ml) were added to different plats and the media were poured into plats. The samples were mixed with media by rotating the plats clockwise and anticlockwise. The plates were incubated at 37°C for 24 hrs. After the completion of incubation period the plats were observed for growth. Colonies were counted using colony counter with the method described by Oyedeji et al. (2010).

All the samples were cultured on MacConkey and S.S agar. MacConkey agar was used as a presumptive medium because it is both selective and differential media for members of Enterobacteriaceae family. MacConkey agar plates were prepared and samples were cultured on it with spread plat method. The plates were incubated at 37°C for 24 hrs. After the completion of incubation period the plats were observed for bacterial growth.

Salmonella-Shigella agar (SS agar) was used for the identification and conformation of the presence of *Salmonella* and *Shigella* species of bacteria. The samples were added to media and were spread on agar plats with the help of spreader. The plates were incubated at 37°C for 24 hrs. After the completion of incubation period the plats were observed for bacterial growth.

Biochemical tests

As mentioned above that *Salmonella* and *Shigella* were identified by using S.S agar while for the identification and conformation of E. coli the following biochemical tests were used.

Triple sugar iron test (T.S.I)

Triple sugar iron test (T.S.I) was used for identification of *E. coli*. As it is based on fermentation of lactose, glucose and gas production so *E. coli* positive samples produced yellow butt and slop cracks or bubble. The media was prepared according to the manufacturer's

instructions and a quantity of 6 ml was poured in each test tube and sterilized by autoclaving at 121°C for 15 minutes.

Media was placed in slop position and allowed to solidify to form butt and slant. After that slant was inoculated with a straight wire loop both in butt and slant, incubated at 37°C for 24 hrs and results were recorded.

Citrate test

It is used for the differentiation of *E. coli* from *Klebsiella*. Both of them give same result on T.S.I but *E. coli* is citrate negative while *Klebsiella* is positive. The test based on ability of organism to use citrate as its only source of carbon and ammonia as source of nitrogen. The media was prepared according to the manufacturer's instructions and a quantity of 6 ml was poured in each test tube.

Media was sterilized by autoclaving at 121°C for 15 minutes. Then media was allowed to solidify, inoculated with a straight wire loop, incubated at 37°C for 24 hrs and observed for results.

Indole test

Indole test is used for the confirmation of *E. coli*. *E. coli* break amino acid tryptophan with the release of indole. The media was prepared according to the manufacturer's instructions and a quantity of 3 ml was poured in each test tube. Media was sterilized by autoclaving at 121°C for 15 minutes, inoculated with test organism, incubated at 37°C for 24 hrs. About 0.5 ml of Kovacs reagent was added to each test tube. If red layer form on surface it meant that the test is positive otherwise the test is negative.

Results

Total bacterial count

A total of 100 water samples were analyzed for bacterial count. These samples were collected from different sources in 20 water distribution systems. In each water distribution system, samples were taken from source point and point of use. The data was analyzed and the range and mean value were calculated (Table 1).The source wise percentage of water above or within WHO guidelines are shown in Fig. 1.

Table 1. The range and mean values for bacteriological quality of water samples taken from different sources at Danish Abad, Peshawar.

Water source	Total no. of samples	Range cfu/ml	Mean values cfu/ml	No. of samples within WHO guideline value (<10 cfu/ml)	No. of samples excess to WHO guideline value (>10 cfu/ml
Source point (tube wells)	20	0 - 4.4×10^1	2.16×10^1	6 (30%)	14 (70%)
Point of use (houses)	70	0 - 2.8×10^4	4.33×10^3	4 (5.7%)	66 (94.3%)
Self bores	10	4×10^1 - 4×10^3	1.3×10^3	0 (0%)	10 (100%)

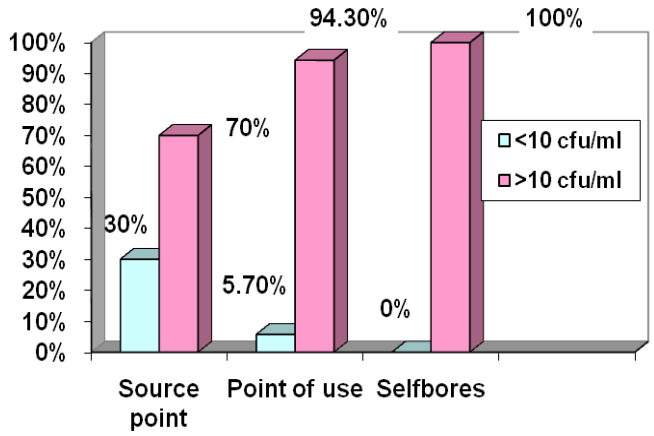

Fig. 1: Source wise percentage of water above or within WHO guidelines (<10 cfu/ml)

Distribution of *Shigella, Salmonella* and *E. coli* in water samples

Out of 100 samples 32 were positive for *Shigella*, 8 for *Salmonella* and 10 for *E. coli* as shown in Table 2. The percent wise distribution is shown in Fig. 2.

Table 2. Distribution of different targeted pathogenic bacterial species in water samples collected from different source points and point of use at Danish Abad, Peshawar.

Organism	Total samples	Positive samples	Percentage
Shigella	100	32	32%
Salmonella	100	8	8%
E. coli	100	10	10%

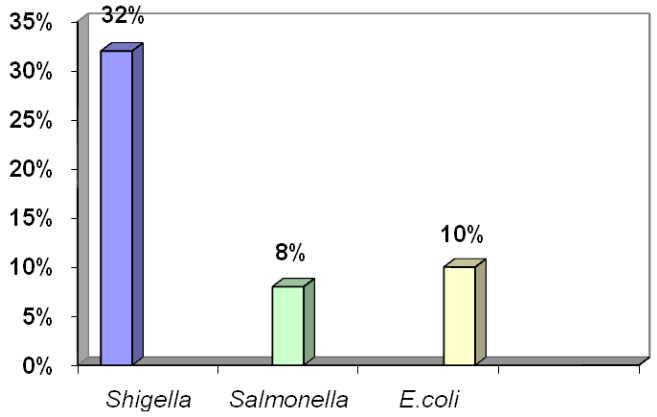

Fig. 2: Distribution of different targeted pathogenic bacterial species in water samples collected from different source points and point of use at Danish Abad, Peshawar.

Source wise distribution of bacteria in water

At source point out of 20 samples 2 were positive for *Shigella* (10%), *Salmonella* and *E. coli* were negative in all samples. The distribution of different targeted pathogenic bacterial species at water source point is shown in Table 3 and Fig. 3.

Table 3. Distribution of different targeted pathogenic bacterial species at water source point.

Organism	Total samples	Positive samples	Percentage
Shigella	20	2	10%
Salmonella	20	0	0%
E. coli	20	0	0%

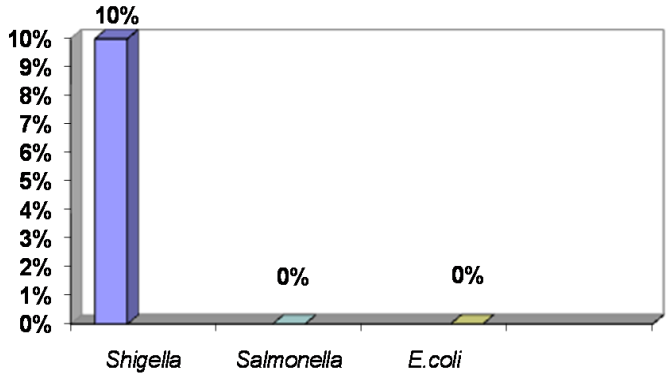

Fig. 3: Distribution of different targeted pathogenic bacterial species at water source point.

At point of use out of 70 samples 30 were positive for *Shigella* (42.8%), 8 for *Salmonella* (11.4%) and 8 for *E. coli* (11.4%). The distribution of bacteria at point of use is shown in Table 4 and Fig. 4.

Table 4. Distribution of different targeted pathogenic bacterial species at point of use.

Organism	Total samples	Positive samples	Percentage
Shigella	70	30	42.8%
Salmonella	70	8	11.4%
E. coli	70	8	11.4%

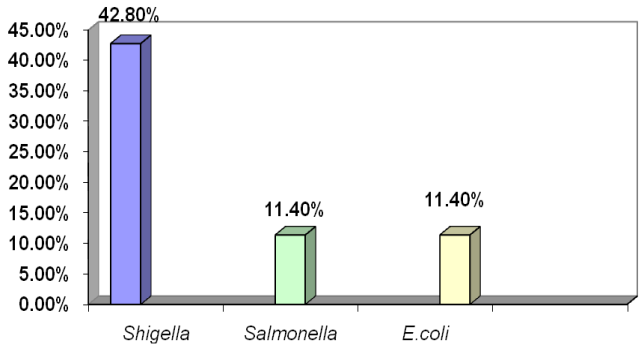

Fig. 4: Distribution of different targeted pathogenic bacterial species at point of use.

Totally 10 samples were collected from the houses which have house bores, out of which 2 samples were positive for *E. coli* (20%) and no sample was positive for *Salmonella* and *Shigella* as shown in Table 5 and Fig. 5.

Table 5. Distribution of different targeted pathogenic bacterial species in individually used house bores of Danish Abad, Peshawar.

Organism	Total samples	Positive samples	Percentage
Shigella	10	0	0%
Salmonella	10	0	0%
E. coli	10	2	20%

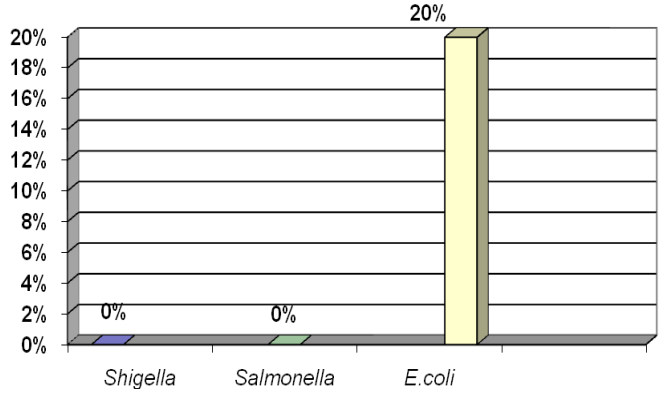

Fig. 5: Distribution of different targeted pathogenic bacterial species in individually used house bores of Danish Abad, Peshawar.

Discussion

A total of 100 water samples were collected from different localities of Peshawar and analyzed for total bacterial count at point of use and source points. The results were compared with the WHO standards. At source point 70% and at point of use 94.3% of the samples were above WHO values. These findings are in close agreement with the findings of Prasai et al. (2007) who reported it 71.1% at source point and 89.5% at point of use. The mean value at source point was low (2.16×10^1 cfu/ml) as compare to point of use (4.33×10^3 cfu/ml). At point of use the results of the present study were closer to Adetunde and Glover (2010) who reported it 4×10^4 cfu/ml, but contradict at source point (1.65×10^4 cfu/ml) which may be due to contamination that usually occur due to defective joints, back siphonage, rusted pipelines crossing over the sewage pipes and low/high pressure in the pipelines.

The results of the bacteriological analysis of drinking water from Danish Abad showed that most drinking water sources are contaminated with pathogenic bacteria. The bacterial species identified were members of the *Enterobacteriaceae* family. The most prevalent species was *Shigella* (10% at source point and 42.8% at point of use), *Salmonella* and *E. coli* (11.4% on point of use while no sample was positive at source point). A similar study was conducted by Prasai et al. (2007) who reported a different prevalence rate for these pathogens with highest prevalence of *E. coli* (26.4%) which may be due to the failure of the disinfections of the raw water at the treatment plant, as this species usually comes from fecal contamination through defective sewage system. This way of contamination of drinking water was also reported by Haydar et al. (2009).

Conclusion

The study concluded that most of the water samples were unfit for drinking because it has high TPC value and does not meet WHO standards. The health risks are associated with consumption of water due to presence of pathogenic bacteria *Shigella*, *Salmonella* and *E. coli*. Further study is required to know about other pathogenic organisms like fungi and viruses. Water must be purified by chlorination, filtration or boiled before use.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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