

BACTERIOLOGICAL QUALITY OF DRINKING WATER IN MARDAN, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The present study was aimed to isolate and identify micro-organisms load of drinking water of Mardan city, Khyber Pakhtunkhwa Pakistan. A total of 27 samples of drinking water were collected from different locations of the study area. Total Plate Count was determined by pour plate method, while total coliforms, total fecal coliforms and *E. coli* were determined by multiple tube fermentation method. Of the total collected samples, 17 (62.96%) samples were contaminated with either one or more than one type of microorganisms. The results of most probable number test showed that 13 (48.15%) samples were unsatisfactory. It was concluded that the water should be treated before consumption for drinking purpose. Regular assessment of the water quality is recommended as regular monitoring of the water quality for improvement not only prevents disease and hazards but also checks the water resources from becoming further polluted.

Key words: Drinking water, Coliform bacteria, *E. coli*, diseases, health issues.

INTRODUCTION

Water is essential to sustain life, and a quality (adequate, safe and accessible) supply of water must be ensured to all. Water is unsafe for human consumption when it contains pathogenic or disease-causing microorganisms (Fernández *et al.* 2007). The high prevalence of diseases such as diarrhoea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Brown *et al.* 2006, Ullah *et al.* 2014a). The most dangerous form of water pollution occurs when faecal

contaminants enter the water supply. Pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *E. coli* being shed in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into ground water.

Microbiological analysis of water is a method of analyzing water to estimate the numbers of microbes present and, if needed, to find out what sort of microbes they are (Anderson *et al.* 2005). It is very expensive and time consuming process to test for all the possible microbial pathogens in water, so a single group of microorganisms that

came from the same source as human pathogens is used to indicate the presence of pathogens (Mahbub *et al.* 2011). According to WHO, more than 5 million people die annually due to water borne diseases. Of these total mortalities, more than 50% are attributed to microbial intestinal infections (Cabral 2010). Coliform bacteria are used as microbiologic indicators for water quality. Freedom from contamination with fecal matter is the most important parameter of water quality because human fecal matter is generally considered to be a greater risk to human health as it is more likely to contain human enteric pathogens that are agents of diarrhoea (Esomonu *et al.* 2012).

The current study was designed to investigate the microbiological quality of drinking water collected from different locations of Mardan city, Khyber Pakhtunkhwa, Pakistan. As the microbiological condition is very important for water quality, the aim of this work was to find out the possible cause of fecal contamination that might cause severe waterborne fatal disease.

MATERIALS AND METHODS

Sample collection

A total 27 water samples were randomly collected from different locations of Mardan city. About 500 ml of water were aseptically collected in pre-sterilized screw capped bottle and transported to the laboratory at earliest using ice box. The analysis was carried out in the Food Microbiology Laboratory of Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan. The sampling was carried out during the period of March to August 2013.

Total viable count

Total Viable Count was determined by pour plate method as described. One ml of the sample from the three dilutions (10⁻¹, 10⁻² and 10⁻³) were taken and added to each duplicate Petri dish. Plate

Count Agar was added to each Petri dish and incubated at 35°C for 48±2 h, after incubation colonies were counted by Colony Counter and result was expressed as CFU/ml.

Total Coliform bacteria/fecal Coliform bacteria

The most probable number (MPN) of total coliforms bacteria were determined by multiple tube fermentation technique. Separate sets of 10 tubes of Lauryl Tryptose Broth (LTB) were prepared and inoculated each set of 10 tubes of double strength LTB with 10 ml of the water sample. These LTB tubes along with inverted Durham tubes were incubated at 35 ± 0.5°C for 24 and 48±2 h after inoculation. Tubes were examined for gas production at the end of 24/48 h incubation. Gas production was measured by gas displacement in the inverted vial and also effervescence produced when the tube was gently shaken. Positive tubes with gas formation and turbidity were sub-cultured into BGB (Brilliant Green Lactose Bile broth and E.C. Broth having 10ml broth with inverted Durham tubes by means of 3 mm loop). All BGB tubes were incubated at 35 ± 0.5°C for 48±2 h and E.C. Broth tubes at 44.5±0.01°C for 24±6 h and examined for gas production. Total coliforms and fecal coliforms were calculated from MPN tables.

***Escherichia coli* O157:H7**

EMB Agar was used for the enumeration of *E. coli*. All the tubes of E.C. broth showing gas were sub cultured on EMB agar plates and incubated at 35°C for 18-24 h. Positive plates contained typical colonies with green metallic sheen colour were inoculated on PCA slants (plate count agar) and incubated at 35°C for 18-24 h and were identified biochemical and also by kits (*E. coli*O157:H7 latex test reagent kit Pro Lab. Canada).

Determination of antibiotic susceptibility

The anti-bio grams of each isolate was determined on freshly prepared, dried up Nutrient

agar (Oxoid) using the agar-disk diffusion method. The tested antibiotics included (Oxoid): amoxicillin (30 µg), Ampicillin (30 µg), Cephradine (30 µg), Cefotaxime (10 µg), Cefixime (30 µg), Ciprofloxacin (10 µg), Ofloxacin (10 µg), Gentamycin (10 µg). The *E. coli* ATCC 8739 was used as standard for quality control of the susceptibility tests. Based on zones of inhibition, interpreted using the criteria recommended for by NCCLS (2002), isolates were classified as either sensitive (S), Intermediate (I) or resistant (R). Pure cultures of organisms were enriched in nutrient broth and incubated at 37°C to a turbidity of 0.5 Macfarland standards. The nutrient agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30 min to allow for diffusion of the antibiotics and the plates were incubated inverted at 37°C for 24 h. Results were recorded by measuring the zone of inhibition and comparing with the NCCLS interpretive performance standard for antimicrobial disk susceptibility testing.

RESULTS AND DISCUSSION

The water samples collected from different locations of District Mardan were tested for TPC (total plate count), TC (total coliforms), FC (fecal coliforms) and *E. coli*. Of the total analyzed 27 samples, 10 were found fit and 17 samples were unfit for drinking purpose (Table 1). The TPC of Bicket Ganj water sample was found the highest one 676 cfu/ml. The TPC of Muqam chowk water sample was the lowest one (64 cfu/ml) and found fit for drinking purpose. The TPC of Bank road 340 cfu/ml, Irum colony 78 cfu/ml, Shekh malton town 70 cfu/ml, Bag-e-Ram Road 66 cfu/ml, New adda 128 cfu/ml, Bijli ghar 190 cfu/ml, Chatoo chowk 348 cfu/ml, Shamse road 520 cfu/ml, Eid

ghah road 68 cfu/ml, Gharib market 310 cfu/ml, Gaju khan 184 cfu/ml, Shaheedano bazaar 72 cfu/ml, Kachaehri chowk 76 cfu/ml, PRC chowk 86 cfu/ml, Dosehra chowk 1456 cfu/ml, Mohallah Tarkan 230 cfu/ml, Parhoti road 84 cfu/ml, Dosehra chowk 188 cfu/ml, Station korona 560 cfu/ml, pirano dag 346 cfu/ml, sabzai mandi 340 cfu/ml, and TPC of Baghdada was 155 cfu/ml. Table 1 reveals bacteriological analysis of drinking water of the study area.

The *E. coli* form bacteria of Bank road water sample showed the highest value 23 MPN/100 ml and new adda, Shaheen market, Dosehra chowk showed the lowest value of 2.2 MPN/100ml. The *E. coli* form bacteria of Irum colony, Shekh malton town, Bag-e-ram road, Eid ghah road, Pakistan chowk, Muqam chowk, Pohan colony, Gaju khan, Shaheedan bazaar, Kachaehri chowk, PRC chowk, Parhoti road, Dosehra chowk, Baghdada were <1.1 MPN/100ml. New adda, Shaheen market Dosehra chowk 2.2 MPN/100ml, Bijli ghar, Garib market, Station korona, Sabzai mandi 3.6 MPN/100ml, Chatoo chowk 5.1 MPN/100 ml, Bicket ganj 12 MPN/100ml, Shamse road 6.9 MPN/100ml, and Mohalla Tarkan 9.2 MPN/100ml, Pirano Dag 16 MPN/ 100ml.

The Fecal coliform bacteria of 20 samples were found in range while that of 7 samples were found out of range. The Bicket ganj and Piranp dag water samples had the highest value 9.2 MPN/ 100ml and that of bijli ghar water sample has lowest 1.1 MPN/100ml. Chatoo chowk, station Korona and Sabzi mandi has 2.2 MPN/ml, Mohallah Tarkan has 3.6 MPN/100ml.

Bacteriological studies were conducted for the determination of *total* and *Fecal coliforms* bacteria present in the drinking water. Coliforms bacteria are present in the soil, water, human colon or animal waste. The detection of coliform contamination in drinking water indicates flaws in treatment systems as untreated sewage water being directly flowed into the fresh water canals.

Secondly, drinking water during distribution process gets contamination from sewage system (Ullah *et al.* 2014b), as presence of Fecal coliform and *E. coli* indicate water may be contaminated with human or animal wastes and intake of

contaminated water pose serious health risks of waterborne diseases to the community (Aziz *et al.* 2013). Table 2 is showing WHO standards regarding drinking water for bacteriological factors.

Table 1. Bacteriological analysis of drinking water of the study area.

SN	Location	TPC (CFU/ml)	Coliform Bacteria (MPN/100 ml)	Fecal Coliform (MPN/100 ml)	<i>E. coli</i>
1	Bank Road	340	23	<1.1	-ve
2	Irum Colony	78	<1.1	<1.1	-ve
3	Shekh Maltoon Town	70	<1.1	<1.1	-ve
4	Bag-e-Ram Road	66	<1.1	<1.1	-ve
5	New Adda	128	2.2	<1.1	-ve
6	Bijli Ghar	190	3.6	1.1	-ve
7	Chatoo Chowk	348	5.1	2.2	-ve
8	Bicket Ganj	670	12	9.2	+ve
9	Shamse Road	520	6.9	<1.1	-ve
10	Eid Ghah Road	68	<1.1	<1.1	-ve
11	Pakistan Chowk	158	<1.1	<1.1	-ve
12	Muqam Chowk	64	<1.1	<1.1	-ve
13	Pohan Colony	82	<1.1	<1.1	-ve
14	Garib Market	245	3.6	<1.1	-ve
15	Shaheen Market	310	2.2	<1.1	-ve
16	Guju Khan	184	<1.1	<1.1	-ve
17	Shaheedano Bazar	72	<1.1	<1.1	-ve
18	Kachaehri Chowk	76	<1.1	<1.1	-ve
19	PRC Chowk	86	<1.1	<1.1	-ve
20	Dosehra Chowk-1	456	2.2	<1.1	-ve
21	Mohallah Tarkan	230	9.2	3.6	-ve
22	Parhoti Road	84	<1.1	<1.1	-ve
23	Dosehra Chowk-2	188	<1.1	<1.1	-ve
24	Station Korona	560	3.6	2.2	+ve
25	Pirano Dag	346	16	9.2	-ve
26	Sabzi Mandi	340	3.6	2.2	+ve
27	Baghdada	155	<1.1	<1.1	-ve

Bicket ganj, Station korona and sabzi mandi water samples were found contaminated with *E. coli O157:H7*, while the rest of water samples were found free from it.

Table 2. WHO standards of drinking water.

SN	Bacteriological parameters	WHO Standards
1	Total Plate Count (CFU/ml)	<100
2	Total Coliform Bacteria (MPN/100ml)	<1.1
3	Total Fecal Coliform Bacteria	<1.1
4	<i>Escherichia coli</i> (O157: H7)	Nil

Table 3. Antibacterial susceptibility test of the isolated *E. coli*.

SN	Name of sample	Capacity of Antibiotics	Zone of Inhibition in mm		
			<i>E. coli</i> -8	<i>E. coli</i> -24	<i>E. coli</i> -26
1	Amoxicillin	30 µg	S ^a	S	S
2	Ampicillin	30 µg	S	I ^c	S
3	Cephadrin	30 µg	R ^b	I	S
4	Cefotaxime	10 µg	S	S	S
5	Cefixime	30 µg	I	S	R
6	Ciprofloxacin	10 µg	S	S	S
7	Oflaxacin	10 µg	S	S	S
8	Gentamycin	10 µg	S	R	I

^a Sensitive; ^b Resistance; ^c Intermediate

Antibiotic susceptibility pattern of pathogenic isolates (*E. coli*) of this study is presented in Table 3, showing the inhibition zones produced by preselected antibiotics. All isolates (*E. coli*) were sensitive to Amoxicillin, Cefotaxime, Ciprofloxacin and Oflaxacin. *E. coli* 8 and *E. coli* 26 were sensitive, while *E. coli* 24 show intermediate result to Ampicillin. *E. coli* 8 was resistant, *E. coli* 24 was intermediate and *E. coli* 26 was sensitive to Cephadrin. *E. coli* 8 was intermediate, *E. coli* 24 was sensitive and *E. coli* 26 was resistant to Cefixime. *E. coli* 8 was sensitive, *E. coli* 24 was resistant and *E. coli* 26 was intermediate to Cefixime.

This study showed that all water samples found polluted with total and fecal coliform bacteria mostly with large number of colonies, which is a major health risk of waterborne diseases for the citizens and particularly children under five. Besides, no residual chlorine is detected in any of water samples analyzed, which indicated poor treatment mechanism at the source level and, of course quality is unsafe too at the end consumer level as water passes through deplorable distribution network and unsanitary conditions.

Keeping in view the high level of contamination of drinking water in this part of our country, it is essential to educate the local people and make them aware of the ill effects of

deteriorating water quality (Ullah *et al.* 2014a). This will improve their level of understanding and practices towards drinking water quality. The local communities should be made aware of treating water before use such as through boiling; chlorination and use of ceramic filter technology as well as improving the overall sanitation within the residential areas should be instituted.

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