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Microbiological Analysis of Drinking Water of Gravity Flow Water Scheme Abbottabad, Pakistan

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Abstracts

Since pure drinking water is good for health, it must be assessed for bacterial contamination. This research was conducted on the gravity flow water scheme of District Abbottabad, Pakistan. Pakistan. Twenty water samples were collected in different areas of city. Physiochemical analyses including pH, temperature, and chlorine residue showed that all water samples were according to prescribed values by the World Health Organization. This research work aims to analyze the total viable count (TVC), Total Coliform Bacteria (TCB), Total fecal Coliform Bacteria (TFCB) present abundantly in drinking water. The results showed that highest TVC values 5.6020 log cfu /100ml and lowest were 3.7781 log cfu/100ml, TCB were found in the range 250>2.0MPN/100ml, TFCB were found in the range 25>1.1MPN and *E.coli* were found in all water samples except sample-11. The water samples analysis data indicated that 100% water samples were unfit due to high TVC values, 85% samples were unfit due to higher (TCB) and 45% TFCB than permissible limits and 75 % samples were found to be contaminated with *E. coli*. *Escherichia coli*, *Klebsiella oxytoca* and *Pseudononas aeruginosa* were identified based on morphology, gram staining and conventional biochemical tests and the identification was further verified by API 20 E kit.

Key word: API 20E kit, Microbiological Analysis; Drinking Water; Gravity Flow Water Scheme

Introduction

The project of gravity flow water scheme is located in the district Abbottabad, Pakistan. The project initiated on 15th April, 2011 and was completed in September of 2013. The total expenditure to complete this project was 4.28 billion rupees out of which 3.7 billion rupees were provided by the Japanese government, and the rest was provided by the KPK provincial government [1].

The plan provided the city of Abbottabad with drinking water and benefited 216,353 people. Among them, 65% of the population lives in urban areas and 35% of the population lives in rural areas. To store water, four different pools were built in Namly Maira, Bandi and Bagh [1].

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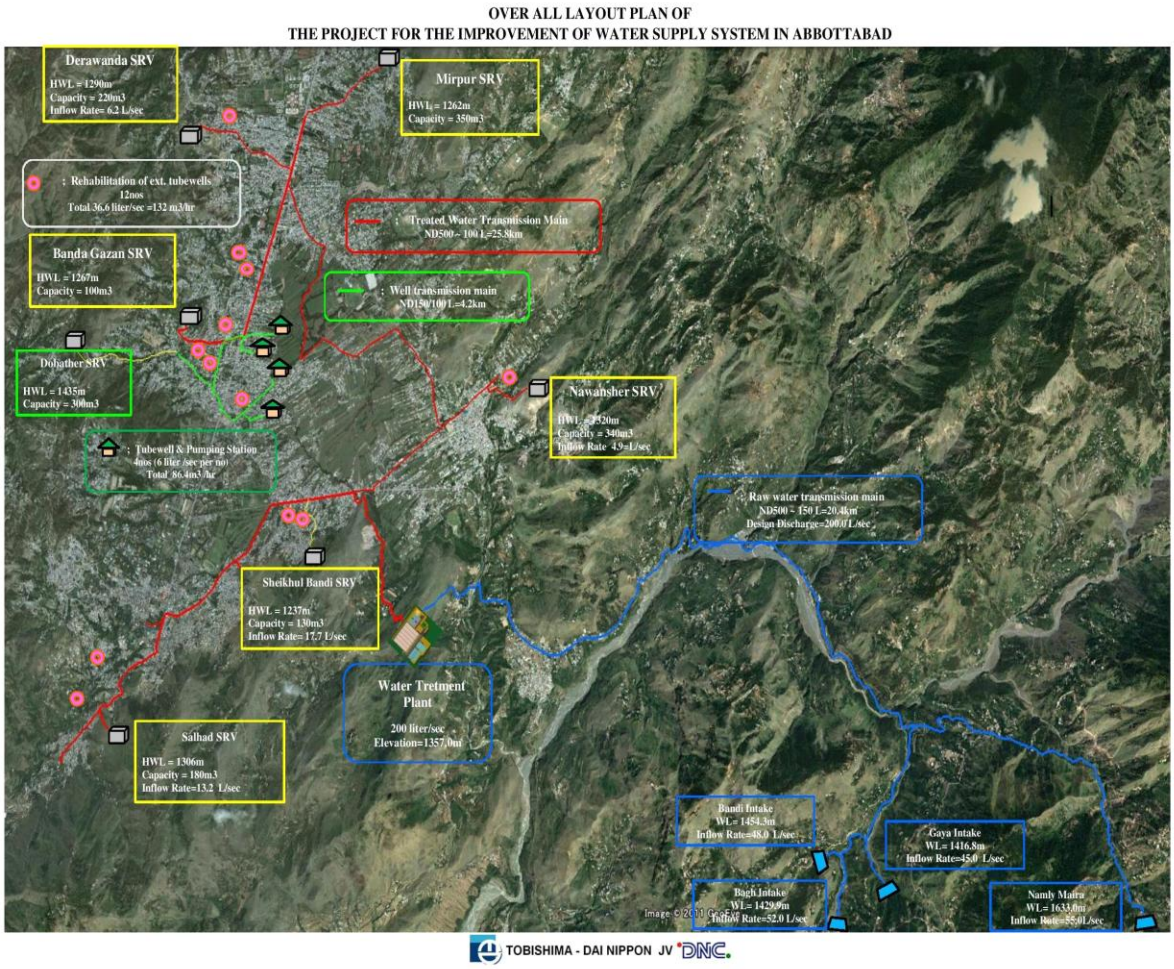
To purify water, a treatment plant was built in Chuuna Kala Pul Abbottabad to purify 38,000 gallons of water (200L/S) per day. Drinking water is supplied by this plant in different areas of Abbottabad, including Sheikul bandi, Salhad, Newansher, Mirpul, Derawanda and Danda. In addition to the camp tube well pumping station is used to provide, drinking water in the Dobass region.



More than 3.4 million people are affected each year due to contaminated water as reported by the WHO. The chief culprits of causing major diseases and deaths around the world are water-borne pathogens [2]. The feces of humans or animals and chickens are major causes of water contamination. The, fungi, viruses, protozoa, and worms are generally secreted by feces.

The intestinal diseases and some others infectious diseases are mainly the result of using contaminated water [2]. According to the latest report of the Pakistan Water Research Council, the water quality of most water supply projects in Pakistan exceeds the drinking water quality standards set by the World Health Organization [2].

Materials and Methods

Site description

Abbottabad is a city of KPK provience of pakistan. It covers an area of nearly 1969 square kilometers; the city of Abbottabad is surrounded by Serbian mountains on all sides.

Sample collection

20 water samples at different sampling points in Abbottabad were collected. They were put into 150ml sterile polyethylene bottles. The pH and temperature of the sample was noticed at the sample collection site, and kept them in an ice box and transported them to the Quaid-i-Azam University Soil Microbiology Laboratory for further processing

Enumeration of total viable count

1 ml water sample was taken from each collected sample and added to a serial dilution of 9 ml normal saline. Then inoculated 0.1 ml of the dilution on the nutrient agar plate and the three un-inoculated plates, and incubated at 37°C for 24 hours. After incubation, visible colonies formed on each plate, but no visible colonies appeared on the un-inoculated plates. Then colony counter was used to count colonies on plates that contain 30 to 300 colonies, and the results were presented as cfu/100 ml.

Multiple Tube method

Multi-tube method was used for determining the most probable number (MPN) of coliforms and fecal coliforms and *Escherichia coli*. The test was carried out according to the standard protocol [3]. Sterile 10ml and 1ml water samples were added to test tubes containing 10ml and 5ml double-strength Mac Conkey broth respectively.

In addition, 0.1ml of water sample was added to a test tube containing 5ml of MacConky Broth of a single concentration. All test tubes contain sterile inverted Durham test tubes and were incubated at 37°C for 48 hours.

Positive samples were sub-cultured into 10ml single tube of brilliant green bile broth with inverted Durham tubes and10ml Peptone Water to find out presence of fecal Coliform and E.coli and the tubes were incubated at 44◦C for 24 hours. The MPN of fecal Coliform and *E.coli* was determined according to the standard WHO guidelines.

Procedure for isolation of selected colonies

1ml of coliform and fecal coliform cultures on the MacConkey agar plate was inoculated. Sterile spreader was used to evenly distribute the cultures on the plate. The plate was kept in the inverted position in incubator and keeps growing at 37°C for 24 hours. After culturing for a period of time, colonies of different colors were formed, and some suspicious colonies were selected from every plate to precede further work.

Identification of microorganism

For confirmation of suspected colonies Gram staining technique, conventional biochemical test and API-20E kits was used [4].

Result

During November to December, the pH, temperature as well as residual chlorine content of drinkable water samples were measured. The maximum pH value of drinking water was 9.3, the minimum pH value was 7.43, and the average value was 9.36. The highest temperature of drinking water samples was 18 C°, the lowest temperature was 9.7 C° and their average value was 12.675 C°. The maximum value of residual chlorine was 1.7 ppm, the minimum value was 0.4 ppm and their average value was 1.025 ppm. (Table 1)

Physiochemical parameter

Table-1: Physiochemical character of water samples

NOV TO DEC	Parameter	MINMIUM	MAXIMUM	MEAN	STD DEVIATION
	pH	7.43	9.3	9.365	0.3969
	Temp(^{O}C)	9.7^{O}C	18^{O}C	12.675 ^{O}C	4.4364 ^{O}C
	Chlorine residual	0.4ppm	1.0ppm	1.025ppm	0.3339ppm

Total viable count

The total viable counts of individual drinking water samples were relatively high, which means that these water samples are not safe for drinking .The maximum number of total viable bacteria was 5.6 log cfu/100ml, and the minimum log was 3.8 log cfu/100ml. eighteen drinking samples have a medium value (Figure 1). Therefore, the result is not significant and does not meet the requirements of the WHO

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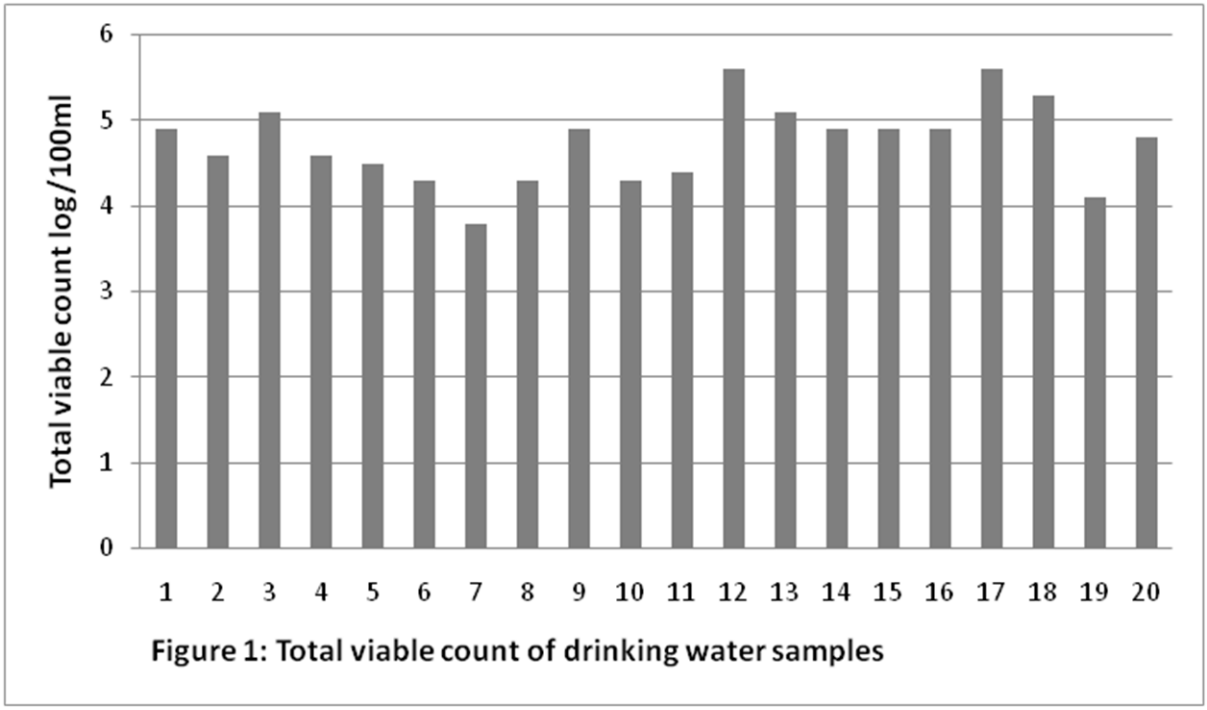

Figure 1: Total viable count of drinking water samples

Most probable number of drinking water samples

The MPN/100ml values of coliform, fecal coliform and *E. coli* were analyzed from water samples from various locations in Abbottabad, such as residences, schools, hotels and restaurants, and distribution lines. The MPN/100ml value of various drinking water samples is greater than the standard protocol set by WHO, so the water is not suitable for community use.

The higher MPN/100ML value of total Coliform (250,35,50) were reported in Serbund Student Berger Restaurant, Storage Water Govt Girl High School Abbottabad and House No :261 and lower value (2,2,2) in House No:k494, House No:50/2 and Bandkhou Abbottabad as presented in fig.2A

Fecal coliform (35, 25, 25) in House No: 261, Govt Girl Hostel No: 1 and Serbund Student Berger Restaurant were higher MPN/100ML and lower value of fecal coliform were reported in seven localities in House No:k494, House No:204, House No:247, Storage Water Govt Girl High School Abbottabad, House No:50/2 and Bandkhou Abbottabad as presented in fig.2B

The highest value MPN/100ML of *E.coli* (25,9,9) of drinkable water samples were reported in House No: 261 Boland Hotel and Serbund Student Berger Restaurant and lowest value in House No:247 was shown as presented in fig.2C .

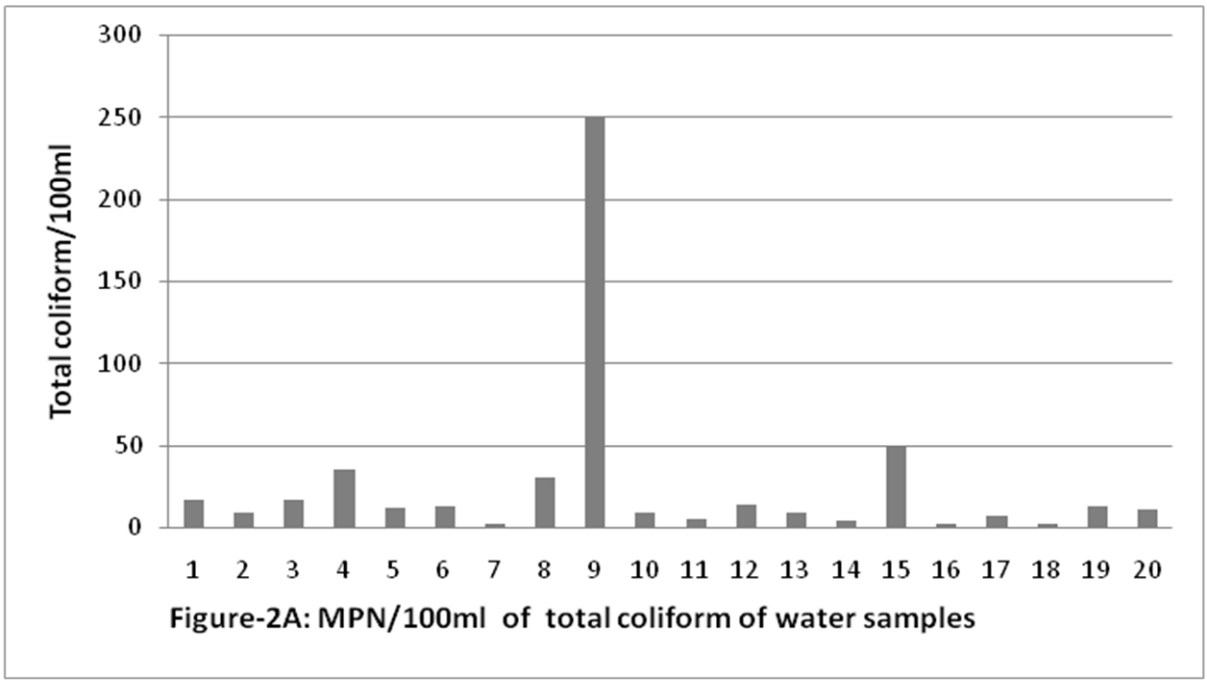

Figure-2A: MPN/100ml of total coliform of water samples

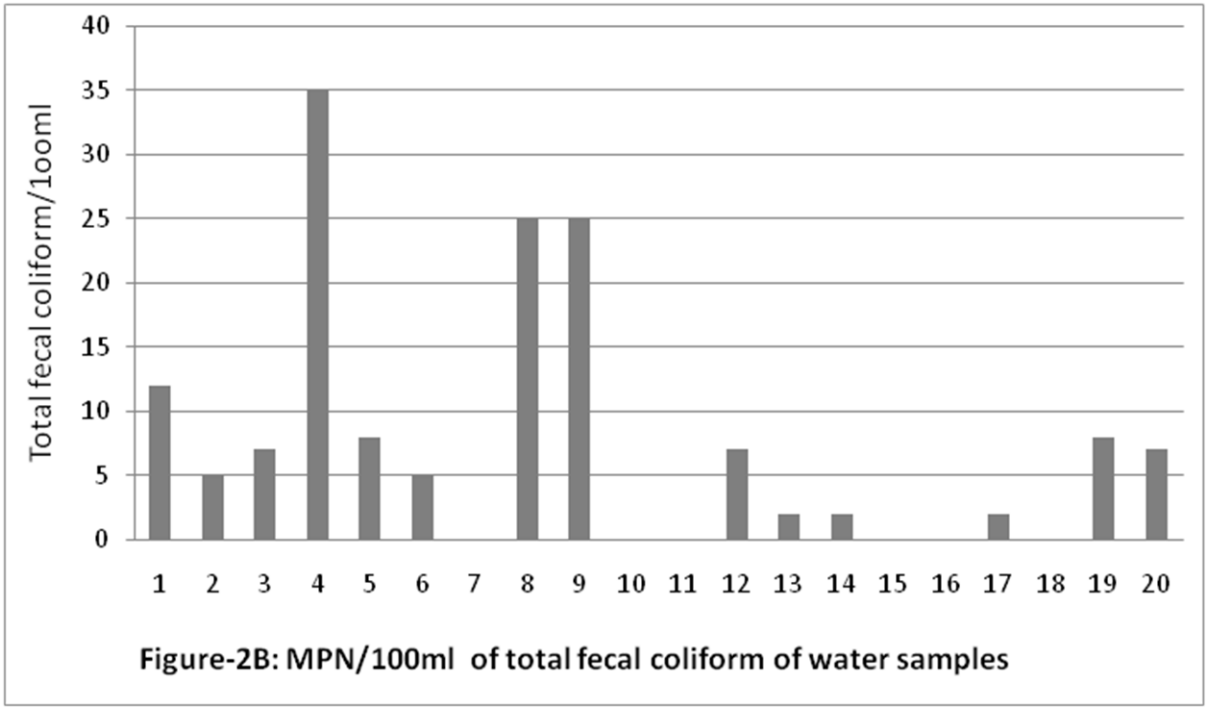

Figure-2B: MPN/100ml of total fecal coliform of water samples

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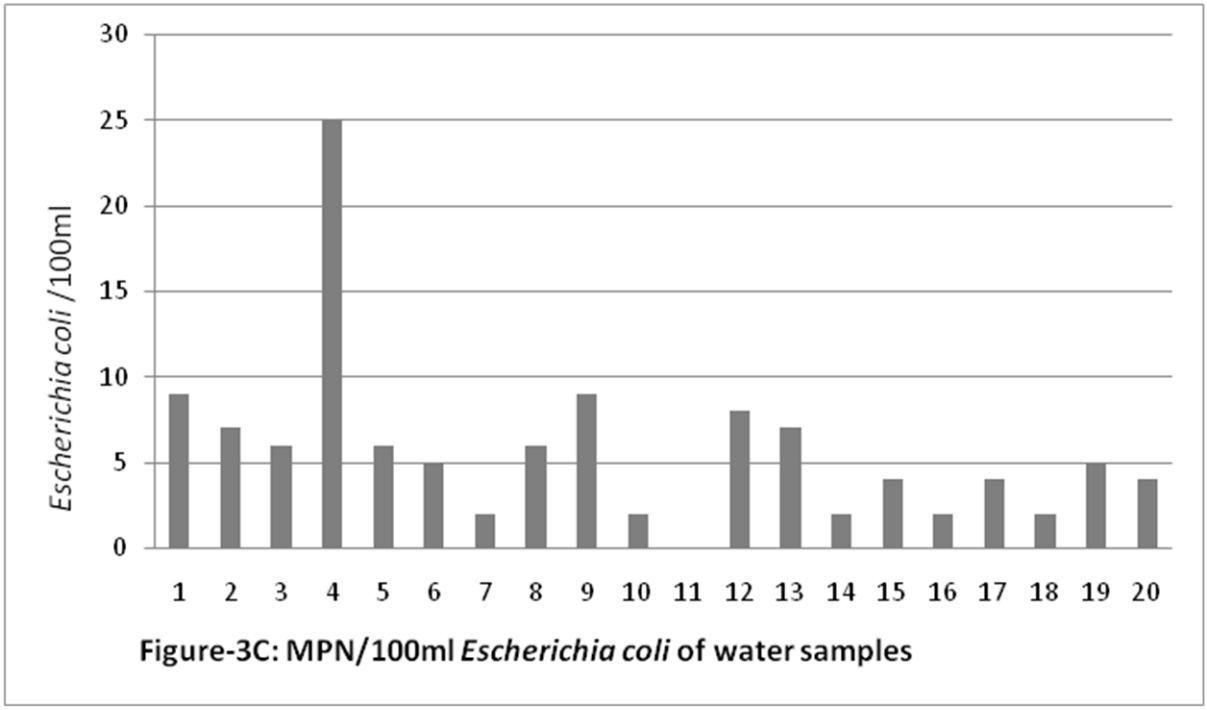


Figure-3C: MPN/100ml *Escherichia coli* of water samples

Isolation and identification of waterborne Pathogens

Eescherichia coli, Pseudomonas aeroginosa, Klebsiella oxytoca isolated from drinking water by using different microbial technique including Gram staining, Oxidase, Mobility and further bacteria verified by API-20E kit.

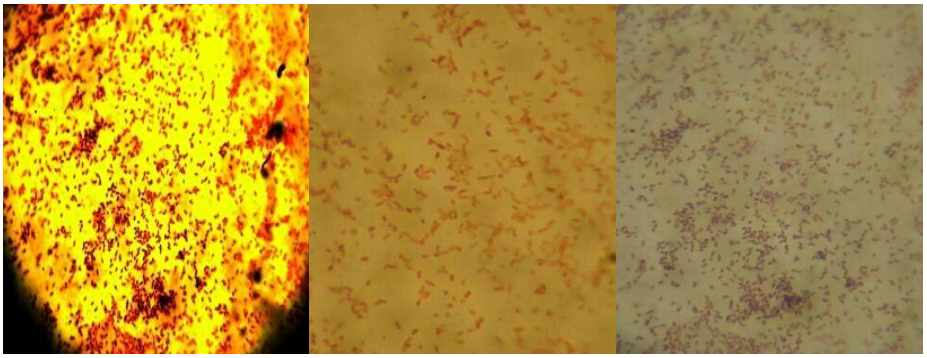
Table 2: Biochemical identification of pathogenic Bacteria

S/N 0	Full name of substrate	*Escherichia coli*	*Pseudomonas aeroginosa*	*Klebsiella oxytoca*
1	Gelatinase	+	+	_
2	Voges-Proskauser	_	_	+
3	Indole	_	_	+
4	L-tryptophane Deaminase	_	_	_
5	Urease	_	+	+
6	Hydrogen Sulphide	_	_	-_
7	Citrate	_	+	+
8	Decarboxylase Ormithine	_	_	_
9	Lysine Decarboxylase	_	_	+
10	Arginine Dihyrolase	_	+	_
11	Ortho-Nitro Phenyl-BD Glactopyranoside	_	_	+

12	L-Arabinose	_	_	+
13	Amygdaline	_	_	+
14	L-Melibiose	_	_	+
15	D-Saccharose	_	_	+
16	L-Rhamnose	+	_	+
17	D-Sorbitol	+	_	+
18	Inositol	_	_	+
19	Mannitol	_	_	+
20	Glucose	+	+	+
21	Gram staining	_	_	-
22	Oxidase test	_	+	_

Table 3: Morphological identification of pathogenic Bacteria

S/N0	Morphological character	*Escherichia coli*	*Pseudomonas aeroginosa*	*Klebsiella oxytoca*
1	shape	rod	rod	rod
2	Motility	motile	motile	non-motile
3	Color appearance	pink	colorless	creamy mucoid
4	Baceria gram staining	negative	negative	negative



Escherichia coli Pseudomonas aeruginosaKlebsiella Oxycota

Discussion

According to world health organization standard, total viable count and coliform should not be greater than 100 cfu/ml [5].

It has been observed that the total viable bacteria counts of water samples collected from different sampling points in Abbottabad are very high. 5.6020 log cfu/100ml compared with water tape and tube well 4.0791 log cfu/100ml of Peshawar drinking water [6, 7].

The results of MPN/100ml drinking water samples included 17 out of 20 samples (85%) contaminated with total coliforms, 11 (45%) contaminated with fecal coliforms, and 15 (75%) contaminated with Escherichia coli Contamination (similar report by Abdul et al., 2009) All 120 (100%) samples in Sukkur City were contaminated with total coliforms, and 98 (82%) samples were contaminated with heat-resistant E. coli.

The same research work was done to determine microbial contamination of individual drinkable water sources for example ground, well and river water reported *E.coli* and fecal coliform [8] are the main contaminants of 67% of total water sample. Comparable results were also reported by another study [9].

The present study showed that drinking water samples were more contaminated. They consist of number of bacteria but the most abundant bacteria found in different drinking water samples were commonly *Escherichia coli*, *klebsiella Oxytoca* and *Pseudomonas aeruginosa*. Another study made a similar report [10]. The results showed that analysis of drinking water samples from the Kathmandu Valley and they identified 238 strains of intestinal bacterial isolates. Among them, *Escherichia coli* was 26.4%, *Pseudomonas aeruginosa* was 6.3%, *Klebsiella* 5.4%, *Shigella* 4.0%, *Vibrio cholerae* 1.0%, Salmonella was 3.0%.

Conclusion:

Microbiological analysis of drinking water samples revealed that all water samples were contaminated with microbes such as coliforms, fecal coliform and *E.coli.* It is responsible to cause diseases in community and kept extra burdon on the society as a result the area cannot progress properly. So government should have to pay attention towards these problems and revises the policy and do work for it

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Conflict of interest

The author declares that there is no conflict of interest in the publication of this paper.

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