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Bacteriological analysis of well water in distract Swat KPK Pakistan

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Abstract

The aim is to study Bacteriological analysis of well's water in district swat. For bacteriological analysis, different methods were used like Pour plate technique, MPN (Most Probable Number) and various agar and broth mediums for the growth and identification of coliform and fecal coliform bacteria like MacConkey agar medium, MacConkey broth medium, Eosin methylene blue agar, mannitol salt agar. Fourteen samples collected from the open well as well as from closed well were first tested for coliform by inoculating the water into 10ml broth medium having inverted Durham tube. The samples showing gas production after incubation were taken as positive. They were then subjected for culturing on an agar medium to identify the fecal Coliforms in them. The highest TPC value in open wells was 612 CFU/ml in Sirsinai while the lowest TPC value was that in Totanobandai 182 CFU/ml. *E.coli* are positive in some sample include Totanobandai, Sirsinai, Kalakaly, Bandai and Saidu Sharif. While closed wells indicate the highest TCF value 521 CFU/ml, 4.5 coliform and 1.2 fecal coliform MPN/100ml in Shah dherai and lowest is 26 CFU/ml. So the people using well water in Swat villages are at high risk for different infections like Cholera, dysentery, Salmonellosis, GIT infections, etc. because of high contamination in water. The current study shows a high level of contamination in these well's water samples from different pathogenic microorganisms.

Keywords: Well water, bacterial contamination, coliform

1. Introduction

The importance of water in human life can't be over-emphasized. Water is much important for irrigation, houses, industries, cultural aims & for the maintenance of biosphere [1]. Water is the most precious source of liquid for the maintenance of living organisms. The worldwide amount of water that is good for human consumption on ground & surface water is very low quantity 0.3%, whereas on the other hand 2.97% comes from ice caps & glaciers and the remaining 97% of water come from oceans which are not good for health and drinking [2]. Apart from its use for direct human consumption, water is also linked to the provision and quality of ecosystem service. In homes, water is used for cooking, drinking, bathing and cleaning purposes. However, water is also available for other uses which will be a subject of concern throughout of the world, and especially in the rural and semi-urban areas Pakistan. Pure water for drinking is the ultimate thing for better and healthy life and this is one of the natural rights of all humans. As we know that Fresh and pure water are limited sources in most regions throughout the globe. Due to climatic changes, urbanization, overpopulation in the next hundred years, it will be greatly reduce [3]. Due to contamination through waste mainly feces by animal and human sources water can spread potentially contagious diseases during water supplies. Worldwide but mostly in the developing countries are at more risk of the outbreaks of waterborne illnesses. Following are the micro-organism that extremely lethal to human when present in drinking water include *Yersinia enterocolitica*, *Salmonella*, *Shigella*, *Campylobacter*, some viruses which include *Hepatitis A Virus*, *Hepatitis E Virus*, and *Rota Virus* and some parasites such as *Giardia lamblia*, *Entamoeba histolytica* and many more [4]. Regrettably, in developing countries like Pakistan the feature of water which is used for drinking purposes are increasingly contaminated by different organisms and dangerous for use of humans. Water is contaminated due to increasing in population, increasing the number of industries, disposal of waste water and many more drinking water sources.

Due to which many countries in Asia are coming up with the problem of increase in nutrient materials and organic compounds inside water bodies which are used for drinking purposes because of the emission of infectious domestic waste and wastes from industries into these water bodies [3, 5]. Throughout the world mortality rate due to the bad quality of water in a year is more than people dying due to other violent reasons which include war etc and which is probably about 26% of entire deaths are due to diseases which are highly spreadable having a causative agent are infectious microorganisms [6]. Pakistan also facing the above problem while pure water is available for about 40-60 percent of total population [6]. In Pakistan work based on water, microbiology is not so much done and it is a most ignoring task for research. Some studies which were done in different areas of Pakistan shows that the water used for drinking purposes are highly contaminated. Approximately 70% Pakistanis depend on the above source of water for domestic use. Because of contaminants and microorganisms, many of the people have no access to pure water because of the improper drainage system and bad water supply lines. Due to consumption of contaminated water, can cause serious many diseases like Cholera and Typhoid. Contaminated water is the main cause of 40 percent of all deaths and 30 percent all hospitalized peoples in Pakistan [7]. Research about water microbiology is a neglected topic here in Pakistan so we designed the present study to determine the present status of drinking water quality in different areas of Swat which is bacteriological analysis.

1.1 Study Objectives

- Sampling of water from different areas in swat
- Conducting Bacteriological analysis of water.

2. Materials and Methods

2.1 Study area and sample collection

Swat is a district of Khyber Pakhtoon Khwa which is situated on the N-W of Pakistan and is very lovely and productive district of KPK which is located in longitude 72.07 °North-East through 73.00 °East and latitude 34.09 °North through 35.56 °North. The borders are touched in the north with Chitral and Gilgit District, at the south is Buner, at east, is Shangla while at the West is Dir and Malakand agency. The population of Swat in 2014 was approx 1.161 million out of which approx 86% villages and towns while the remaining 14 percent were in cities. The entire number of villages are 1250 and 145041 are families dependent on farming [8]. Samples of water from 15 wells will be collect for water quality analysis i.e. bacteriological analysis. Water from the well are the major sources for bathing, drinking and for many other household purposes in such area. The majority of the well water we are going to study will be privately owned and are normally open for general public. About 1/4 of the number of the wells in our study are covered while the remaining is not covered. By the use of 10-15 liter container, we will draw water samples from these wells. Which will be tie-up immediately to the cover of the well? In some instant where it will not be applied, then we will draw the water from well with small containers. All the sides of a covered well are well-organized and cemented while certain herbs are grown on all sides of the uncovered wells. Incomplete sterile bottles (about 500g) will collect water samples from wells. With the help of a strong metallic string of about 500 grams of weight, we will draw water from uncovered wells. Keep the weighted bottles, remove their caps aseptically and then be dipped in 1-2 meters of wells. As these bottles come out of the wells we need to

screw capped it immediately and to reduce the risk of contamination [9-11].

2.2 Bacteriological surveying

We find out the total bacterial count by pouring plate technique by using standard methods. We also use MacConky agar medium for the purpose of culturing of bacteria from our samples. The MPN index method will be used for the total Coliform Count. Acid and gas are produced when incubated at 37 °C for 48 hrs when the result is positive on MacConkey broth. Pour plate tech is used for the fecal coliform count by using Eosin Methylene Blue medium. *E. coli* strains will be showing greenish metallic sheen clusters on Eosin Methylene Blue agar & for the confirmation, we also the ability of an organism to ferment lactose at 44.5 °C. on the other hand *Aerobacter aerogenes* will appear as large pinkish mucoid colonies on media. We subject all of our samples to Multiple Tube Test for finding out of the most probable number (MPN) of fecal coliform & Coliforms. We add hygienically, (1) 50-milliliter amount and (5) 10-milliliter amount water to bottles and tubes which will contain 50 milliliter and 10 milliliters which means that MacConkey Broth Medium (Oxoid) is of double strength. Then five 1ml volumes of our collected water samples to tubes which will contain 5 ml of single strength MacConkey Broth Medium. All of our these tubes and bottles in the experiment will contain inverted Durham tubes which will be sterilized before putting them in tubes and bottles. Then we incubate it on 37 °C for 48 hours in the incubator. The bottles or tubes after incubation, which showed acid and gas production will be considered positive for coliforms [7-10]. Due to the separation of these positive bottles and tubes from our samples Most Probable Number (MPN) of Total Coliforms will be determined by considering standard probability table for the finding of Total Coliforms. All the bottles and tubes from our samples which are positive for Total Coliforms, we will subculture them into 10 ml of single strength MacConkey Broth having inverted sterilized Durham tubes and 5 ml of Peptone water to find out the presence and absence of fecal coliforms. Then we will incubate these tubes at 44 °C for 24 hours in an incubator. The tubes which show acid and gas production and indole production will take as positive for Fecal Coliforms. From, the number of these positive tubes we will calculate the MPN of Faecal Coliforms by the table as for Total Coliforms. For confirmation of bacteria, we will subculture the tubes with a fashion in fresh medium and incubate them for 24 hours at 44 °C, Confirmation of fecal coliforms was done by subculturing the positive culture of presumptive coliforms into tubes containing 5 ml broth with MacConkey Broth Purple and an inverted Durham tube. These tubes were kept at incubation at 44 °C, and then a drop of “positive coliform” was added to it and incubation continued for 48 hours. If again there was acid and gas production, it “confirmed” presence of “faecal coliforms” from the Table of MPN (51.6) read off the most probable number of coliform per 100ml. This gave the confirmed coliform count [11].

3. Results

Fourteen samples were collected from different sources (open wells, closed well) in swat area for bacteriological analysis. From the present study, we conclude that open wells are contaminated with coliforms and fecal coliforms like *E.coli*, *Shigella*, *Salmonella* and *vibrio cholera* etc. For finding the fecal coliform we subjected them to single strength MacConkeys broth in a tube having 10 ml of broth and 5 ml

of peptone. These tubes also have inverted Durham's tube for identification of gas production by which we recognized them as positive. Then we inoculate our water samples those which were positive for coliform incubated them for 24 hours in 44 °C. After incubation, those tubes shows gas production are taken as positive. Those which were positive for coliform all shows a positive result for fecal contamination which means this water were mainly contaminated through fecal route. They are contaminated with these fecal coliforms because of the exposure to the outer environment and showed positive results due to leaking and other sources of wastes from humans and other animals. Bacteriological analysis is shown in table 1, the highest TPC value was 612 CFU/ml in Sirsinai while the lowest TPC value was that in Totanobandai 182 CFU/ml. Other TCP values are 68 CFU/ml, 428 CFU/ml, 458 CFU/ml, 201 CFU/ml, 503 CFU/ml samples taken from Galoch, Kala kaly, Bandai, Kanjo and Saidu Sharif respectively. Sirsinai drinking water sample 19 coliform bacteria MPN/100ml, 8.2 fecal coliform bacteria MPN/100ml. others Totanobandai have 8.1 coliform and 1.7 fecal coliform

MPN/100ml, Galoch have >1.1 coliform and >1.1 fecal coliform MPN/100ml, Kalakaly have 30 coliform and 23 fecal coliform MPN/100ml, Bandai have 7.2 coliform and 4.1 fecal coliform MPN/100ml, Kanjo 2.1 coliform and 1.1 fecal coliform MPN/100ml, Saidu Sharif 5.1 coliform and 3.6 fecal coliform MPN/ml. *E.coli* are positive in some sample include Totanobandai, Sirsinai, Kalakaly, Bandai and Saidu Sharif. Present study about closed wells indicate the highest TCF value 521 CFU/ml, 4.5 coliform and 1.2 fecal coliform MPN/100ml in Shah dherai and lowest is 26 CFU/ml, >1.1 coliform and >1.1 fecal coliform MPN/100ml Samai, others Dewlai 31 TPC, >1.1 coliform and >1.1 fecal coliform MPN/100ml, 42 TPC in Maira, >1.1 coliform and >1.1 fecal coliform MPN/100ml, Manja 67 TPC, >1.1 coliform and >1.1 fecal coliform MPN/100ml, Amonkott 56 TCP, >1.1 coliform and >1.1 fecal coliform MPN/100ml, Qalagy 417 TPC, 8.6 coliform and 3.1 fecal coliform MPN/100ml. Shah dherai and Qalagy are *E.coli* positive.

Table 1: Bacteriological Analysis of closed wells

S.N	Sample Location	Total Plate Count (CFU/ml)	Coliform Bacteria (MPN/100ml) They are rod-shaped Gram-negative non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35–37 °C. Coliforms can be found in the aquatic environment, in soil etc	Fecal Coliform Bacteria (MPN/100ml) A fecal coliform (British: faecal coliform) is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium. Coliform bacteria generally originate in the intestines of warm-blooded animals. Its also used as an indicator of sanitary quality of water and food.	<i>E. Coli</i>
1	Totano Bandai	182	8.1	1.7	+ve
2	Galoch	68	<1.1	<1.1	00
3	Kala kaly	487	30	23	+ve
4	Sirsinai	612	19	8.2	+ve
5	Bandai	458	7.2	4.1	+ve
6	Kanjo	201	2.1	1.1	00
7	Saidu Sharif	523	5.1	3.6	+ve

Table 2: Bacteriological Analysis of closed wells

S. N	Sample location	TPC (CFU/ml)	Coliform bacteria (MPN/100ml)	Fecal Coliform bacteria (MPN/100ml)	<i>E.coli</i>
1	Deowlai	31	<1.1	<1.1	00
2	Shah dherai	521	4.5	1.2	+ve
3	Maira	42	<1.1	<1.1	00
4	Samai	26	<1.1	<1.1	00
5	Manja	67	<1.1	<1.1	00
6	Amonkott	56	<1.1	<1.1	00
7	Qalagy	417	8.6	3.1	+ve

4. Discussion

Swat is a well-known district of KPK Pakistan where tourist visits due to its beautiful sceneries and to whom visitors called Singapore of Pakistan. We design a study for its water analysis the study is about only well waters we choose two types of wells i.e. open wells which are not covered and closed wells mainly in homes which they cover them for protection reasons. The villagers totally dependent on well water for drinking and other domestic uses. Therefore we design study to analyze the well waters whether it have bacterial contamination or not.

We collect 14 samples from different wells in different villages of swat district i.e. from closed wells and from open wells. For analysis of contamination, we performed tests to find out the total coliform and also then fecal coliform. After collection of our samples in sterile bottles within 24 hours, we brought them to the lab for performing different tests. First, we find out the most probable number by making serial

dilutions of our samples. After serial dilutions, we cultured them on different media for confirming the coliforms in these water samples. The media used for finding out the coliform was MacConkys broth having double strength and single strength agar. These media were in liquid form i.e. broth present in tubes. 50ml of double strength broth were taken in tubes and about 10ml of our water sample were inoculated in this media. In other tubes, we have 5ml of single strength broth and in this tube, we inoculate about 1ml of our water sample. These tubes have also Durham tubes which were put inverted in tubes by us. After inoculating our sample we incubated these tubes in 37 °C for 24 hours. After incubation for 24 hours, those tubes showing gas and acid production were taken as positive. In our open well water samples 5 out of 7 samples were positive showing gas production so identified as these samples have contamination of microbes which were Totanobandai showing about 8.1 coliforms (MPN/ml), Kala kalay showed 30 coliform, Sirsinai showed

19 coliform, Bandai show 7.2 coliform and Saidusharif well water have 5.1 coliform. While closed well have only 3 positives for coliform out of 7 samples. They include Qalagy having 8.6 coliforms in these water, Shah derai well water have 4.5 coliform and Amankott well water have 9.2 coliforms.

Those samples which showed positive results for coliform which are discussed above were subjected for analyzing fecal coliform to find out whether they were contaminated through fecal route or not. For finding the fecal coliform we subjected them to single strength MacConkeys broth in a tube having 10 ml of broth and 5 ml of peptone. These tubes also have inverted Durham's tube for identification of gas production by which we recognized them as positive. Then we inoculate our water samples those which were positive for coliform. And incubated them for 24 hours in 44 °C. After incubation, those tubes shows gas production are taken as positive. Those which were positive for coliform all shows a positive result for fecal contamination which means these water were mainly contaminated through fecal route. Villages from which water samples were taken and show positive results for fecal coliform are Totanobandai water have 1.7 fecal coliforms, Kala kalay 23, Sirsinai 8.2, Bandai 4.1, Saidusharif 3.6, Qalagy 3.1, Shah derai 1.2 and Amankott 6.1 fecal coliforms.

The most contaminated water samples were from Kala kalay which shows 30 coliform and 23 fecal coliform and Sirsinai water samples have 19 coliforms while 8.2 fecal coliforms. This means those peoples living in these villages are at high risk for water-borne diseases.

Those samples which show a positive result, then different test were performed for identification of different microorganisms like Mannitol salt agar is used for *Staphylococcus aureus*, Nutrient agar used for culturing of different bacteria present in our samples, Eosin methylene blue medium used for identification of *E.coli* which shows greenish colonies on such media.

If people using such contaminated water for drinking purpose continuously are at high risk for diseases like *Campylobacteriosis*, *Cholera*, *E. coli* infections, *Dysentery*, *Salmonellosis*, *Respiratory infections*, *Hepatitis A virus* and many other *Gastro intestinal infections*. Villagers living in such villages included in our study shows mostly sign and symptoms of *Cholera*, *Salmonellosis*, *Dysentery* and other *Gastrointestinal infections*. We can say that it is due to such contaminated water use for drinking and other domestic purposes. To avoid the risk of contamination of well water, we need that wells dug must be covered properly. Water must be boiled before use for drinking purposes & filtration must be a good practice to prevent the incidence of water-borne diseases.

5. Conclusion

The current study it can be concluded that a high level of contamination is present in these water wells from different pathogenic microorganisms. To avoid the risk of contamination of well, we need that dogged wells must be covered properly and Water must be boiled before drinking and even for other purposes in addition to as a good practice to prevent the incidence of water-borne diseases. Better a thousand times careful than once dead.

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