

Isolation and Characterization of Total Coliform Bacteria from Drinking Water of District Shangla, Khyberpakhtunkhwa, Pakistan

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Abstract

Original Research Article

The current study was carried out in district Shangla of Khyber Pakhtunkhwa, Pakistan. A 100 ml of water sample was collected in sterilized bottles and labelled and shifted to Microbiology lab at Veterinary Research & Disease Investigation Center Abbottabad for further analyses. Water samples ($n=252$) from three different sources viz; spring, Water storage tanks and water supply lines) ($n=84$) water samples from each source were processed. About 69 samples (27.38%) were found positive for total coliform bacteria and 183 samples (72.62%) were negative. In spring water study, 9 samples (10.71%) were found positive and 75 (89.29%) were negative, whereas from storage water tanks 30 samples (35.72%) were positive and 54 (64.28%) were negative for the total coliform bacteria. From 84 tap water samples, 30 (35.72%) were positive and 54 (64.28%) were negative for the total coliform bacteria. The Colony Forming Unit (CFU) from Tape and storage tank water samples was more than 300 while *Escherichia coli* was absent in spring water samples. Moreover, positive samples of spring water differ significantly ($p>0.0382$) from water storage tank ($p<0.1104$) and tap water ($p<0.866$). The spring water samples were found more suitable for drinking purpose than tap water and storage tank samples. Most sites met WHO recommended standard microbiological parameters. To certify well health for the people, proper disinfection should be adopted through chlorination or other appropriate methods to provide drinking water.

Keywords: *Escherichia coli*, water microbiology, source, Colony Forming Unit.

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INTRODUCTION

Water is one of the most significant constituent for life on earth. In oceans, there is about 97% of water, which is not fit for drinking. Overall, 3% only is considered as fresh water, in which glaciers and ice caps constituent 2.97% of water. Only 0.03% water is available as surface and ground water for human use [1]. For good health; clean drinking water is the basic need and right of every living organism on earth inclusive of human beings [2].

Universally, drinking water pollution by pathogenic bacteria causes the maximum health hazard to humans. Among these *Escherichia coli* is one of the most important pathogen from faecal contamination [3]. *Escherichia coli* are mutually shared nearly by all warm

blooded animals, as well as human beings [4]. *Escherichia coli* enumeration has been used as a constant sign of faecal contamination and shows probable occurrence of entero-pathogenic and toxigenic microorganisms.

Escherichia coli is one of the important cause of a disease in children under the age of two, but events can depend on variable in studying people, age factor, investigative standards and diagnostic therapies [5]. Water should be pure and artistically attractive [6]. The most important factors in which water and water supply systems effect the value of drinking water is by water distribution and subsequent distribution systems of water to users [7].

Due to lack of water supply inadequate and poor drinking standards, there may be different health problems. Mortality rate of infant is high due to unsafe of water. So, the quality of drinking water should be entirely free from pathogenic micro-organisms and chemical element in concentration which cause health effects.

Water is important for life, but majority of people do not have access to clean and harmless drinking water. Water-borne bacterial diseases include dysentery, typhoid fever and cholera.

The risk of impure water quality and water-related diseases is one of the serious community health concerns in developing countries including Pakistan. It is basically due to lack proper research and mainly monitoring the water quality parameters for most of the areas of Pakistan. The most population of District Shangla obtains their drinking water from the spring sources. To date; the quality of drinking water has not been declared by the authorities whether it's suitable for drinking purpose or not. Moreover, it's also necessary to investigate the quality of water at source and at end user points to verify the suitability of water supply channels.

The intensity of drinking water contamination especially for *Escherichia coli* is required at all levels. Therefore, the present study was carried out to determine total coliform bacteria to assess the drinking water quality.

The objectives of the current study were to assess the microbiological analysis of fresh water at District Shangla with respect to total Coliform, *Escherichia coli* and its characterization from different water samples.

MATERIALS AND METHODS

Study area

The current study was carried out in district Shangla of Khyber Pakhtunkhwa, Pakistan.

Samples collection

Water samples were collected from tap water supplies, Storage tanks and springs from various locations in the study area. A 100 ml of water sample was collected in sterilized bottles and labelled and shifted to Microbiology lab at Veterinary Research & Disease Investigation Center Abbottabad for further analyses.

Sample processing

The water samples were subjected to lab analyses within 24 hours of collection. About 1 ml of water sample was taken in 10 ml lactose broth in glass test tubes having Durham tubes and vortexed for 1

minute and left for 30 minutes at room temperature and then tubes were placed in the incubator at 37°C for 24 hours [8]. After 24 hours, each tube was carefully visualised for any growth and gas production.

Isolation of *Escherichia coli*

Isolation of *Escherichia coli* was carried out by streaking a loopful from a positive broth tube on Eosin Methylene Blue (EMB) agar plates and incubated at 37°C for 18-to 24 hours. The growing colonies on EMB agar were observed for any growth and confirmed as *E. coli* if a metallic green sheen present on bacterial colonies.

Plate count

The laboratory procedure includes serial dilutions (10-1, 10-2, 10-3.....10-10) of the sample and culturing these on EMB agar and colony counting.

Microscopy

The gram stained smears from culture plates were prepared and observed under oil emulsion lens (100X). Gram positive organisms were purple in colour and gram negative organisms appeared pink.

Biochemical identification of *E. coli*

The isolated organism with the appearance of sustaining growth was further subjected to biochemical tests viz; MR (Methyl Red), Triple Sugar Iron (TSI) slant reaction, Indole and VP (Voges-Proskauer).

STATISTICAL ANALYSIS

Data was arranged in Microsoft excel sheets and statistical analyses were performed using SAS, 2008.

RESULTS

Water samples ($n=252$) were from three different sources viz; spring, Water storage tanks and water supply lines) were processed. About 69 samples (27.38%) were found positive for total coliform bacteria and 183 samples (72.62%) were negative.

Table-1: Total water samples processed for screening of *Escherichia coli*

Total samples	252	Percentage
Positive sample	69	27.38%
Negative of sample	183	72.62%

Spring Water Samples

About 9 (10.71%) spring water samples ($n=84$) were found positive and 75 (89.29%) were negative for the total coliform bacteria. The CFU/ml dilution for *Escherichia coli* was >300 . The occurrence of total coliform bacteria in the spring water is shown in table 2.

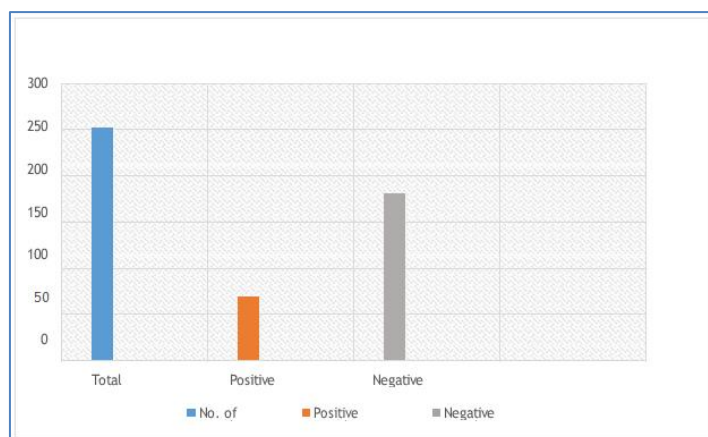


Fig-1: Graphical presentation of total water samples processed for screening of *Escherichia coli*

Table-2: Spring water samples processed for screening of *Escherichia coli*

Parameter	No of samples	%	Total plate count/CFU/ml dilution
Positive for <i>Escherichia coli</i>	09	10.71	>300
Negative for <i>Escherichia coli</i>	75	89.29	<300
Total	84	100	>300

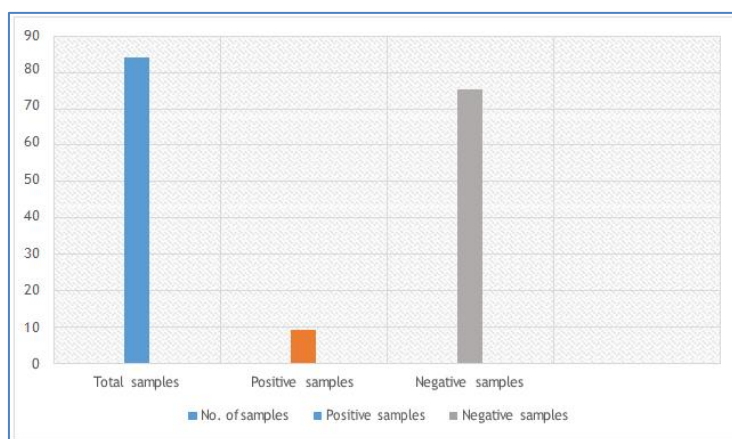


Fig-2: Graphical Presentation of spring water samples processed for screening of *Escherichia coli*

Storage water tanks samples

From 84 water samples of storage tanks 30 (35.72%) were positive and 54 (64.28%) were negative for the total coliform bacteria. The CFU/ml dilution for

Escherichia coli was >300. The occurrence of total coliform bacteria in the water storage tanks is shown in the Table 3.

Table-3: Storage water tank samples processed for screening of *Escherichia coli*

Parameter	No of samples	%	Total plate count/CFU/ml dilution
Positive for <i>Escherichia coli</i>	30	35.72	>300
Negative for <i>Escherichia coli</i>	54	64.28	<300
Total	84	100	>300

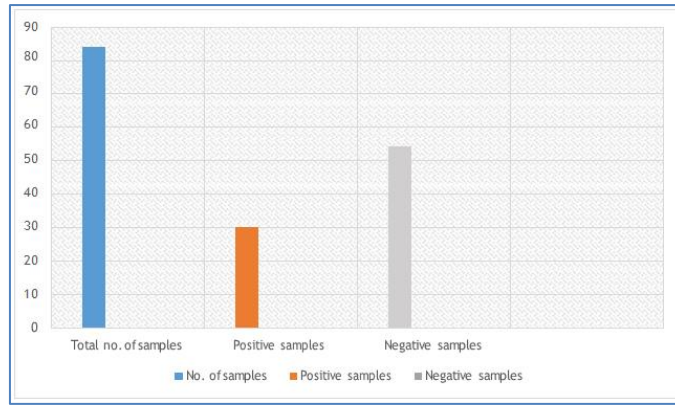


Fig-3: Graphical Presentation of storage water tank samples processed for screening of *Escherichia coli*

Tap water samples

From 84 tap water samples, 30 (35.72%) were positive and 54 (64.28%) were negative for the

total coliform bacteria. The CFU/ml dilution for *Escherichia coli* was >300. The occurrence of total coliform bacteria in tap water is shown in the Table 4.

Table-4: Tap Water samples processed for screening of *Escherichia coli*

Parameter	No of samples	%	Total plate count/CFU/ml dilution
Positive for <i>Escherichia coli</i>	30	35.72	>300
Negative for <i>Escherichia coli</i>	54	64.28	<300
Total	84	100	>300

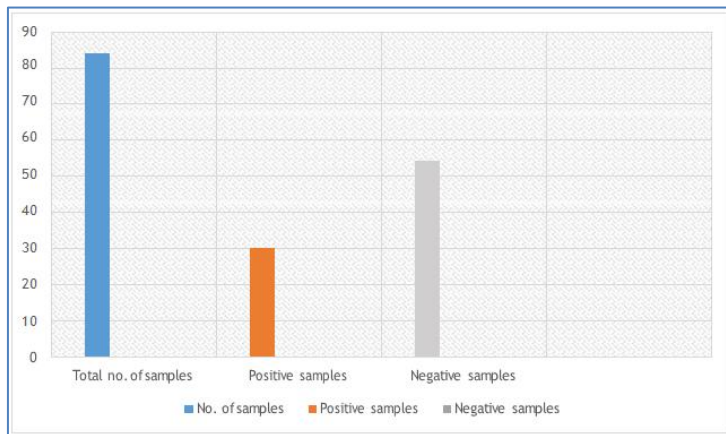


Fig-4: Graphical Presentation of Tap water samples processed for screening of *Escherichia coli*

Morphology of *E. coli* colonies

On EMB agar the growth of colonies were round, small and pink with a green metallic sheen due

to fast fermentation of lactose and creation of strong acids (Fig.5)



Fig-5: Morphology of *E. coli* colonies on EMB Agar

Biochemical identification of *E. coli*

For the recognition of *E. coli* specific biochemical test were used. The isolates obtained from positive samples from the study samples set were found to ferment glucose. The isolates were subjected to

different biochemical tests. Strain showed positive results to indole, methyl red, triple sugar iron whereas; negative results were reported to Voges- Proskauer (VP), Simmons citrate. Biochemical tests results for *E. coli* are summarized in Table 5.

Table-5: Biochemical identification *E. coli* in drinking water of positive samples

Parameter	Indol test	Methyl Red test	Voges Proskauer test	Triple sugar Iron Test	Simmon's citrate test
Spring water	+	+	-	+	-
Water Storage Tank	+	+	-	+	-
Tap water	+	+	-	+	-

DISCUSSION

Globally, many people are dispossessed of this basic necessity. In developed countries it is not severe issue, because 95 % of the population has right to use clean drinking water and 90 % of the people has satisfactory disposal services. Though, in the developing countries the condition is worse, wherever most of the population use contaminated water with undesirable ranks of chemical, toxic, pathogenic microorganisms and suspended solids [9]. It has been projected by United Nations that at least 2.5 billion people have no suitable hygienic system and about 780 million people have no access to harmless drinking water [11]. It has estimated by [11] that globally, about 2.3 billion people are suffering from water associated diseases. About 344 ground water samples was collected from different regions in the country were contaminated; about 65% with total coliform and 35 % with *E. coli* [12]. Then without some earlier treatment, surface water is generally openly used for human drinking, its pollution with total coliform can pose a hazard to the community. A total of 27 water sources were tasted in the Federal Capital Islamabad, in which 74 % samples were polluted with total coliform and 41 % with *E. coli* [13].

One of the finest and most systematically studied free living organisms is the bacterium *E. coli*. *E. coli* is also an unusually different species in which some strain lives as harmless commensals in human and animal intestines. In water bodies *E. coli* is commonly used indicator of faecal pollution. Subsequent ingestion and external contact of bacteria can source harmful health problems from faecal contamination in water. Only non faecal sample that is verified for *E. coli* were the water samples [14].

The examination for total coliform bacteria in drinking water sources for humans in the community of District Shangla has been conducted during current study. A total of 252 samples of water examined, 69 (27.38 %) samples were positive for total coliform bacteria. The water samples were collected from three point sources of water, i.e. spring, water tank and tap water. Out of 252, 84 samples were collected from each point of water. The occurrence of total coliform in

spring source of water is 9 (10.71 %), 30 (35.72 %) in tank water and 30 (35.72 %) in tap water. The examination of total coliform bacteria indicated that the value of water at spring point was better as compared to other two points. Our study is in agreement with the study of [15].

The occurrence of total coliform in the mentioned study revealed that, a total of 17 water samples were collected from different points, i.e. ground water, water storage tank and tap water. Out of those 11.76 % were suitable, 29.4 % were admirable and 5.88 % were doubtful. The isolation of pure culture and consequent identification was done through Gram-staining during current study. Gram-staining colours either purple, in which case they are mentioned to as Gram positive or pink which are known as gram negative.

The characterization of *E. coli* of the positive samples was accomplished through biochemical reaction. *E. coli* gave a positive result to indole, Methyl Red (MR) and Triple Sugar Iron (TSI) and negative to Voges-Proskauer (VP) and Simmons citrate tests. The quality of water for total coliforms ranged from 0 to > 300 MPN/ ml. The limit for total coliform recommended by WHO is 0 MPN/ml [16], which was exceeded in 27.38 % of the water samples during current study. Moderately greater total coliform concentrations in water storage tanks and tap water symbolize the need for instant and actual control methods. Total coliform consist of a wide range of aerobic, facultative anaerobic, Gram-negative, non-spore forming bacilli skilled of growing in the occurrence of comparatively high concentrations of bile salts with the fermentation of lactose and production of aldehyde or acid at 35-37 within 24 hours. The cleanliness and reliability of storage services and supply system serve total coliform as indicators [16].

A total of 72.62 % samples in the study sample set exceeded standard guideline value. The major problems concerning water value in District Shangla is the moderate pollution of potable water sources. The district was also affected with earth quake in 2005 and flooding events in 2010. The basic water and hygiene

setup frequently becomes totally or partially damaged due to submergence in earth quake and flooding events. In the current study, the most common isolate was *E. coli*. For the microbiological value of surface and ground water, *E. coli* has been commonly used as an indicator. The isolation of total coliform mainly *E. coli* from the sources of water is attributable to pollution by animal and human basis and this is of the health importance as these bacteria have mostly been mediator in humans of gastroenteritis [17]. The occurrence of *E. coli* was much found in water storage tank and tap water. The contamination might have come from the point, point of distribution, or leaking water pipes. Water storage tank and tap water must be treated, must have been polluted as a result of non-treatment, or as a results of leaky water pipes, which are submerged in municipal drainage system hence getting contaminated.

Moreover, positive samples from the study sample set of spring differ significantly ($p > 0.0382$) from water storage tank ($p < 0.1104$) and tap water ($p < 0.866$). Based on the total plate count, 27.38 % of the water points were found to be in poor condition for human ingestion.

CONCLUSION

The results obtained from the study reported that the microbiological status of water sampled from different sites of the district is satisfactory. Most sites met WHO recommended standard microbiological parameters. Though, certain sites do not encounter the recommended parameters of WHOM. Many water-borne disease and chronic health problem may be possible to consumption of unsafe drinking water. To certify well health for the people, proper disinfection should be adopted through chlorination or other appropriate methods to provide drinking water.

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