

## Assessment of Heavy Metal Presence, Water Quality, Microbial Load and Health Impact of Tube-well Water in Nalitabari Township of Sherpur District, Bangladesh

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### ABSTRACT

This study aimed to determine water physiochemical parameters, fecal coliform, total coliforms, heterotrophic plate count, arsenic, iron and lead to evaluate the health effect due to the presence of microorganisms and heavy metals. An intensive study was carried out using tube-well water collected from Nalitabari township of Sherpur District in Bangladesh. ~~This study aimed to determine water Physiochemical Parameters, fecal coliform, total coliforms, heterotrophic plate count, arsenic, iron and lead to evaluate the health effect due to the presence of microorganisms and heavy metals.~~ The DO, TDS, salinity, and conductivity, were in the ranges of 4.30 to 7.30, 350 to 792 mg/l, 0.2 to 0.5 ppm, and 715 to 1970 mg/liter and pH values were slightly less or more than permissible value. Due to adjacent to the latrine, 17 tube-well water was contaminated by fecal coliforms and the highest heterotrophic plate count was  $7.5 \times 10^3$  cfu/ml in Ward-8. *E. coli* and *Vibrio cholerae* were identified in 30.56% and 18.06% tube-well water, respectively, resulting diarrhea ~~takes~~ took place in children after drinking. About 6.94% of tube-well water was contaminated with arsenic and 3.25% and 4.5% respondents ~~of the study area~~ were suffering from skin diseases and headache, respectively.

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**Key words:** Tube-well water, Contamination, pH, Heavy metal, Arsenic, Skin disease.

### Introduction

Groundwater from quaternary to recent sediments is the principal source of water for domestic consumption and utilization in industry/ irrigation system in Bangladesh. The shallow alluvial aquifers are consuming water through rainfall and flooding as the static water level in much of Bangladesh ~~is due to its availability~~ within 7 meter of the ground surface year-round. ~~and s~~ Simple suction hand pumps are the dominant water supply technology in Bangladesh (Luby *et al.*, 2008). More than 90% of households in Bangladesh generally use tube-well water for domestic consumption like drinking and cooking purposes. It is a matter of great sorrow that the drinking water is getting polluted with various organic and inorganic matters (Rezania *et al.*, 2015). Depending on availability and the level of groundwater, these tube-wells have been installed in Bangladesh at various depths. It may be insufficient to avoid contamination of the tube-well water with human-pathogenic bacteria due to unfavorable immediate environmental conditions (e.g. the

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distance of tube-wells from latrines or sewage-contaminated ponds or tanks). Despite regular use of tube-well water for drinking, Bangladesh has failed to protect the gastrointestinal diseases caused by water pollution (Islam *et al.*, 2001). Diarrheal diseases are still a leading cause of death of children under 6 years and about 5.2% of all infant deaths occur in Bangladesh due to diarrheal diseases (Feachem and Koblinsky, 1983). Underground water systems of Bangladesh are increasingly vulnerable due to both microbiological contamination and heavy metal pollution, especially by arsenic and iron. Such problems have also been arisen even in developed countries (Hartley, Edwards and Lepp, 2004).

It was found that 41% water of tube-wells was contaminated by total coliforms, 29% by thermo-tolerant coliforms and 13% by fecal coliforms (Saha *et al.*, 2018). About 40% water of shallow tube-wells in Bangladesh were contaminated with human fecal organisms (Knappett *et al.*, 2011, Malla *et al.*, 2018). Coliform bacteria indicate a pathway for more pathogenic bacteria, viruses and protozoan's that can be introduced by anthropogenic activities and poor sanitation. According to World Health Organization (WHO), placing tube-wells at a safe distance from latrines, ensuring that the tube-well has a sound platform without cracks, and that the hand pump is firmly attached, prevent the contamination of fecal coliforms. Every year more than 3.4 million people die as a result of water related diseases, making it the leading cause of disease morbidity and mortality around the world, especially in South-Asia (Souter *et al.*, 2003). From this point of public health, it is highly imperative that potable water supply system should be safe that prevent and control of diarrheal diseases (Motarjemi and Käferstein, 1999; Yager *et al.*, 2006). Drinking water quality among the natural parameters like as Fe, Mn and salinity are matters of concern over large areas in deep and shallow aquifers, and in both urban and rural areas of Bangladesh (Ahmed *et al.*, 2019).

Arsenic exposure through groundwater has been a major public health problem in the Taiwan, Mexico, USA, Mongolia, Argentina, Chile, India and Bangladesh. Worldwide, more than 100 million people have been estimated to be chronically exposed to arsenic from drinking water contamination of high levels of arsenic. The situation is devastating in Bangladesh about 7-11 million hand pumped tube-wells, approximately half have been estimated to supply groundwater with an arsenic concentration more than 50 micrograms/L, which is the maximum level of arsenic allowed in drinking water (Mukherjee *et al.*, 2006). Up to 77 million people in Bangladesh have been exposed to toxic levels of arsenic from drinking water and one in ten has the probability of developing cancer from the arsenic poisoning (Smith, Lingas and Rahman, 2000). The iron contamination in groundwater is one of the most discussed issues because iron (Fe) contamination in groundwater is now a vital problem in Bangladesh (Hug, Leupin and Berg, 2008). According to Anonymous (2004), about 80% of the diseases in developing countries are related to contaminated water and resulting death toll is as much as 10 million per year (Mara and Alabaster, 1995). The improvement of health is not possible without proper sanitation system. Sanitation is one of the major problems in Bangladesh that threat the public health. In this regard, water supply and

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sanitation facilities in terms of quality and quantity are utmost necessary for assessing the living environment of the urban and semi-urban area of Bangladesh.

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The objectives of the study were to investigate the tube-wells water quality and the microbial load of tube well water and their health impact on people of Nalitabari Township of Sherpur District in Bangladesh.

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## MATERIALS AND METHODS

### Study Area

Nalitabari is an Upazila (sub-district) of Sherpur District under the Division of Mymensingh in Bangladesh. It is located in between 25°01' and 25°13' north latitudes and in between 90°04' and 90°19' east longitudes. It is 174.9 kilometer from Dhaka and situated on the bank of the river Bhogai at northern part of Bangladesh. Nalitabari municipality is one of the oldest municipalities in Bangladesh, established at 1st April in 1869. It has an area of 327.61 sq. km with 42,698 households. The study was carried out from June 2016 to April 2017.

### Sampling

Total 72 water samples from 8 locations (9 samples from each ward) were randomly collected and analyzed. The tube-well was continuously pumped for one minute to clear the way of opening and the water samples were collected in a sterile container. All the samples were stored in ice box with proper aseptic technique and transported to the laboratory immediately for experimental analysis. Samples were collected in sterilized bottles and prior to filling; the sample bottles were rinsed two to three times with the water to be collected. The bottles used for collecting samples for metal analysis were filled with acid to keep the pH of the water samples low. Special caution was taken to restrict the overflow of sample water (with acid) from the bottle. The samples were transferred to the laboratory within the six hours of collection (Jidauna *et al.*, 2013).

### Analysis of Physiochemical Parameters

The water quality parameter such as pH was determined by the digital pH meter (Model: pH Scan WP 1, 2 and made in Malaysia). Buffer solution containing pH 4.0 and 7.0 was used to calibrate the digital pH meter. Digital Electrical Conductivity (EC) and Total Dissolved Solids (TDS) meters (Model: HM digital and made in Germany) were used to determine EC and TDS, respectively. Salinity was also measured by it. The Dissolved Oxygen (DO) was determined by digital DO meter (Model: D.46974 and made in Taiwan) where sodium thiosulphate (0.025N) was used as a reagent (Islam *et al.*, 2014).

### Determination of heavy metal

Arsenic, lead and iron were determined by test kit developed by HACH Company, USA.

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### **Heterotrophic plate count (HPC)**

For determination of heterotrophic plate count, 100 micro liter of a tenfold serial dilution of bottled water and 100 micro liters of a tenfold serial dilution of tube well water from samples were transferred and spread onto a plate count agar medium using micro pipette for each dilution. The diluted samples were spread as quickly as possible on the surface of plate with a sterile glass spreader. One sterile glass spreader was used for each plate. The plates were then incubated at 37°C for 24-48 hours. Following incubation, plates exhibiting 30-300 colonies were counted. The heterotrophic plate count was calculated and the result of total bacterial count was expressed as the number of organism or colony forming units per milliliter (CFU/ml) of water samples (Kabir *et al.*, 2015).

### **Total coliform count**

The most probable number (MPN) test for the presence of coliforms in water carried out according to the procedures described by Harley and Prescott (2002). An estimate of the number of coliforms (MPN) can also be done in the presumptive test. In this procedure, 15 lactose broth tubes were inoculated with the water samples. Five tubes received 10 ml of water, another 5 tubes received 1 ml of water and rest 5 tubes received 0.1 ml of water. A count of the number of tubes showing gas production was then made, and the figure was compared to a table developed by American Public Health Association. The number was the MPN of coliforms per 100 ml of the water sample (Hassan *et al.*, 2018).

### **Detection of fecal coliforms**

The positive presumptive cultures were transferred to lactose broth, which is specific for fecal coliform bacteria. Any presumptive tube which showed gas production after 24 (+/-2) hours incubation at 44.5°C (+/-0.2°C) confirmed the presence of fecal coliform bacteria in that tube and was recorded as positive (Manja, Maurya and Rao, 1982).

### **Isolation of pathogenic bacteria**

To isolate specific pathogenic bacteria, the samples were enriched separately with alkaline peptone water (APW) for plating in thiosulfate citrate bile salts sucrose agar (TCBS) medium, with GN (Gram-Negative) broth for plating in *Salmonella Shigella* (SS) agar, with Enterobacteria Enrichment broth- Mossel for plating in MacConkey medium. From each sample 1 ml of water was added with 3 ml of respective enrichment media. All the samples were then incubated at 37°C for 24 hours. After overnight enrichment, the samples were plated in MacConkey, TCBS and SS agar plate separately. All the plates were incubated at 37°C for 24 hours. After overnight incubation, the plates were observed for selective pathogens. For the confirmation of *Escherichia coli*, red/pink colonies from MacConkey agar plates were plated in eosin methylene blue (EMB) agar plates and for the confirmation of *Vibrio cholerae* standard biochemical tests were performed from the yellow and green colonies in TCBS media, respectively (Pavlov *et al.*, 2004).

### **Biochemical test**

Biochemical tests were performed to identify the bacterial flora from different water samples. In this study different Biochemical test (such as- KIA, MIU, CITRATE, VP, OXIDASE, CATALAE, MANNITOL, STARCH, MR, GLUCOSE, LACTOSE, EMB) were performed according to Bergey's Manual of Determinative Bacteriology, (9th Edition, 1994) (Ewalt *et al.*, 1994).

#### **Antibiotic sensitivity test**

Antibiotic susceptibility test was accomplished by disk diffusion method using the commercial antibiotic disk and MHB on Mullar-Hinton agar to assess the susceptibility and resistance pattern of the isolates. For this purpose, 13 different antibiotic discs were used from commercial sources (Oxoid Ltd. England). The selected antibiotics used were Ampicillin, Amoxicillin, Chloramphenicol, Erythromycin, Tetracycline, Gentamicin, Penicillin, Sulphomethoxazole, Kanamycin, Nalidixic Acid, Ciprofloxacin, Streptomycin, Norfloxacin and Azithromycin. The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standard Institute formerly known as NCCLS (Liasi *et al.*, 2009).

#### **Assessment of health impact**

A semi-structured based questionnaire was built to field investigation for evaluating the health impact of the people who used these tube-wells water. Total 400 (200 children and 200 ~~adults~~ adults, among them 50% were male and 50% were female) respondents of the study area was interviewed to determine the health status of the study area (Rakib *et al.*, 2019).

#### **Result and Discussion**

##### **Physiochemical Properties of Water:**

Amount of pH, DO, EC, TDS and salinity contained in the tube well water of 8 different locations collected from Nalitabari Township of Sherpur district, were summarized in Table 1. The pH value of all water samples ~~were~~ was in normal range from 6 to 8.5. pH value observed for all the water samples were slightly less or more than 7 with the average value of 6.8. Lowest value of pH (6.01) was found in Ward- 8 at TW67 and the highest value (7.92) found in Ward- 3 at TW25. In other study, it was reported that the pH of 60% water samples collected from tube-wells in Matlab of Bangladesh were acidic and was lower than recommended by the World Health Organization (Robinson *et al.*, 2011).

The DO, TDS, salinity, and conductivity of water sample were in the ranges of 4.30 to 7.30, 350 to 792 mg/l, 0.2 to 0.5 ppm, and 715 to 1970 mg/liter, respectively. The mean DO contents of all water samples were 5.78 mg/l. The maximum concentration of DO was 7.30 mg/l in the water collected from TW22 (Ward- 3) whereas the minimum concentration found 3.95 mg/l in TW 15 belong to Ward- 2. The values of DO of all water samples were not satisfactory level as the standard value 6.00 mg/l or more of Bangladesh drinking water set by DoE (Alam *et al.*, 2007).

According to International Organization for Standardization, the palatability of drinking water has been rated to its TDS level as follows: excellent, less than 300 mg/liter; good, between 300 and

600 mg/liter; fair, between 600 and 900 mg/liter; poor, between 900 and 1200 mg/liter; and unacceptable, greater than 1200 mg/liter (Beyene, 2015). So, all the value of TDS was in an acceptable level. The result of the study showed that the electrical conductivity (EC) of 50% water samples were within the standard value of drinking water in Bangladesh. The maximum permissible limit of EC in Bangladesh is 1200  $\mu\text{S}/\text{cm}$  (Mebrahtu and Zerabruk, 2011). It was reported that, electrical conductivity (EC) of the drinking water coolers of different teaching institutes in Lahore ranged from 185-362  $\mu\text{S}/\text{cm}$  and was well within the permissible limit of WHO guideline of 400  $\mu\text{S}/\text{cm}$  (Asif *et al.*, 2015). All the tube-wells water of the study area was within the acceptable salinity range where salinity of the fresh water is 0 to 0.5 ppm. There was a significant relationship among the salinity, TDS and EC, in this study it was found when the tube-wells water salinity was 0.5 ppm or more, the TDS and EC value were also high and that was above 1000 mg/l. Another study suggested that most of the physicochemical parameters of ground-water in Rajshahi city were not at the alarming stage (Rasul and Jahan, 2010).

#### **Presence of heavy metals:**

This study revealed that there were a significant association with the arsenic contamination of tube-wells water and deepness of the tube-wells. Among 72 tube-wells, all the tube-wells that contained excessive amount of arsenic have the deepness within 90 feet. Out of seventy-two tube-wells water, sixteen contained more iron (Fe) than the recommended limit set by Bangladesh whereas in Bangladesh, permissible limit of Fe is 0.3-1.0 mg/l, whereas WHO standard level is 0.1 mg/l. About 93.75% of the tube-wells contaminated with excessive iron in which deepness was within 90 feet (Roy *et al.*, 2015). About 12% of the Water sample contains moderate sediment and 76% samples contains no sediment after centrifuge at 10000 rpm. Table 1 showed that there is no contamination of lead found in tube-wells water in the study area. A study reported that more than 60% of the groundwater in Bangladesh contains naturally occurring arsenic with concentration levels often significantly exceeding 10  $\mu\text{g}/\text{l}$  (Bang, Viet and Kim, 2009). The National Drinking Water Quality Survey report (2009) used an estimated national population of 164 million to estimate that 22 million and 5.6 million people are drinking water with arsenic concentrations more than 50  $\mu\text{g}/\text{l}$  and 200 $\mu\text{g}/\text{l}$  (George *et al.*, 2012), respectively but present study showed very little amount of arsenic contamination in the tube-wells water in the study area. Only 6.94% of tube-wells water was contaminated with arsenic more than 50 $\mu\text{g}/\text{l}$  which is the recommended limit set by DoE, Bangladesh. It was found that out of 330 tube wells in Rajshahi city, 72 were found having arsenic levels above the WHO guideline value (0.01 ppm), of which 30 exceeded the Bangladesh drinking water standard (0.05 ppm) (Rasul and Jahan, 2010). ~~There were a significant association with the arsenic contamination of tube wells water and deepness of the tube wells. Among 72 tube wells, all the tube wells that contained excessive amount of arsenic have the deepness within 90 feet. Out of seventy two tube wells water, sixteen contained more iron (Fe) than the recommended limit set by Bangladesh whereas in Bangladesh, permissible limit of Fe is 0.3 1.0 mg/l, whereas WHO standard level is 0.1 mg/l. About 93.75% of the tube wells contaminated with excessive iron in which deepness was within 90 feet (Roy *et al.*, 2015). About 12% of the Water~~

sample contains moderate sediment and 76% samples contains no sediment after centrifuge at 10000 rpm.

**Table 1: Physiochemical properties and presence of heavy metal in tube-well water**

Sample site	Tube well water (TW)	Deepness of the Tube well (feet)	pH	TDS mg/l	Salinity (%)	DO	EC	Arsenic (As)	Iron (Fe)	Lead (Pb)	Sediment (After centrifuge at 10000 rpm)
Ward - 1	TW1	45	6.58	544	0.5	4.30	1460	-Ve	-Ve	-Ve	Moderate
	TW2	60	7.12	792	0.3	7.10	1280	-Ve	+Ve	-Ve	Mild
	TW3	45	7.23	632	0.3	6.25	1135	-Ve	+Ve	-Ve	Moderate
	TW4	75	7.74	556	0.2	5.50	920	-Ve	-Ve	-Ve	No Sediment
	TW5	90	7.19	782	0.2	6.25	975	+Ve	-Ve	-Ve	No Sediment
	TW6	90	6.42	624	0.3	6.65	1070	-Ve	+Ve	-Ve	No Sediment
	TW7	120	6.78	412	0.2	5.55	860	-Ve	-Ve	-Ve	No Sediment
	TW8	135	6.41	669	0.4	5.15	1135	-Ve	-Ve	-Ve	No Sediment
	TW9	150	6.96	424	0.2	4.95	785	-Ve	-Ve	-Ve	No Sediment
Ward- 2	TW10	45	6.97	534	0.2	5.10	935	-Ve	-Ve	-Ve	Mild
	TW11	45	6.69	433	0.5	4.95	1545	-Ve	+Ve	-Ve	Moderate
	TW12	45	7.05	355	0.5	6.20	1960	+Ve	+Ve	-Ve	Moderate
	TW13	105	6.38	475	0.2	4.85	795	-Ve	-Ve	-Ve	No Sediment
	TW14	75	6.22	676	0.3	4.15	1320	-Ve	-Ve	-Ve	No Sediment
	TW15	90	6.12	765	0.3	3.95	1290	-Ve	-Ve	-Ve	No Sediment
	TW16	150	7.33	690	0.2	7.15	1035	-Ve	-Ve	-Ve	No Sediment
	TW17	135	6.55	434	0.2	5.35	885	-Ve	-Ve	-Ve	No Sediment
	TW18	150	7.17	482	0.2	6.45	765	-Ve	-Ve	-Ve	No Sediment
Ward- 3	TW19	60	6.42	593	0.2	5.20	925	-Ve	-Ve	-Ve	No Sediment
	TW20	60	6.98	648	0.3	6.85	1260	-Ve	-Ve	-Ve	No Sediment
	TW21	60	6.25	456	0.4	5.05	1765	-Ve	-Ve	-Ve	Moderate
	TW22	90	7.62	350	0.2	7.30	825	-Ve	-Ve	-Ve	No Sediment
	TW23	90	7.17	488	0.2	6.45	785	-Ve	-Ve	-Ve	No Sediment
	TW24	105	6.02	396	0.2	4.90	765	-Ve	-Ve	-Ve	No Sediment
	TW25	120	7.92	534	0.4	6.75	1135	-Ve	-Ve	-Ve	No Sediment
	TW26	120	6.63	452	0.2	6.40	795	-Ve	-Ve	-Ve	No Sediment
	TW27	120	7.82	745	0.3	5.85	1530	-Ve	-Ve	-Ve	No Sediment
Ward- 4	TW28	60	6.32	675	0.4	4.45	1745	-Ve	-Ve	-Ve	Mild
	TW29	60	6.83	780	0.3	6.90	1340	-Ve	+Ve	-Ve	No Sediment
	TW30	75	6.64	457	0.5	6.35	1925	+Ve	-Ve	-Ve	Moderate
	TW31	105	7.15	455	0.2	5.45	950	-Ve	-Ve	-Ve	No Sediment
	TW32	90	7.31	590	0.3	6.55	1105	-Ve	+Ve	-Ve	No Sediment
	TW33	105	6.88	358	0.2	5.05	715	-Ve	-Ve	-Ve	No Sediment
	TW34	135	6.29	448	0.2	4.85	810	-Ve	-Ve	-Ve	No Sediment
	TW35	120	7.69	725	0.3	6.65	1375	-Ve	-Ve	-Ve	No Sediment
	TW36	120	6.37	675	0.3	5.70	1550	-Ve	-Ve	-Ve	No Sediment
Ward - 5	TW37	75	6.30	695	0.2	5.15	1345	-Ve	-Ve	-Ve	No Sediment
	TW38	60	7.07	455	0.4	6.45	1730	-Ve	-Ve	-Ve	Moderate

	TW39	75	6.32	670	0.3	5.40	1225	-Ve	-Ve	-Ve	No Sediment
	TW40	90	6.43	575	0.4	5.70	1345	-Ve	-Ve	-Ve	Mild
	TW41	105	7.36	552	0.3	7.05	990	-Ve	-Ve	-Ve	No Sediment
	TW42	90	7.27	764	0.2	6.50	1280	-Ve	-Ve	-Ve	No Sediment
	TW43	105	6.53	572	0.2	5.40	1025	-Ve	-Ve	-Ve	No Sediment
	TW44	90	5.98	763	0.4	4.85	1320	-Ve	+Ve	-Ve	No Sediment
	TW45	120	6.18	726	0.3	5.30	1165	-Ve	-Ve	-Ve	No Sediment
Ward-6	TW46	75	6.64	385	0.4	6.55	1905	+Ve	+Ve	-Ve	No Sediment
	TW47	75	7.19	732	0.3	6.15	1455	-Ve	+Ve	-Ve	No Sediment
	TW48	75	6.78	567	0.3	7.10	1290	-Ve	+Ve	-Ve	Mild
	TW49	105	7.45	678	0.3	5.95	955	-Ve	-Ve	-Ve	No Sediment
	TW50	90	5.88	497	0.2	4.55	920	-Ve	-Ve	-Ve	No Sediment
	TW51	90	6.84	675	0.2	5.70	1295	-Ve	+Ve	-Ve	No Sediment
	TW52	105	6.39	725	0.4	6.05	1365	-Ve	-Ve	-Ve	No Sediment
	TW53	90	6.73	467	0.2	6.60	970	-Ve	-Ve	-Ve	No Sediment
TW54	120	6.55	785	0.3	5.35	1390	-Ve	-Ve	-Ve	No Sediment	
Ward-7	TW55	45	6.05	773	0.5	5.60	1970	-Ve	-Ve	-Ve	Moderate
	TW56	75	6.94	780	0.2	6.40	1325	-Ve	-Ve	-Ve	Mild
	TW57	75	7.58	743	0.3	5.05	1195	-Ve	+Ve	-Ve	Mild
	TW58	90	6.89	743	0.2	6.25	1080	-Ve	-Ve	-Ve	No Sediment
	TW59	90	6.36	674	0.3	5.20	1190	-Ve	-Ve	-Ve	No Sediment
	TW60	105	6.65	533	0.2	7.10	885	-Ve	-Ve	-Ve	No Sediment
	TW61	90	7.45	696	0.2	5.75	1365	-Ve	-Ve	-Ve	No Sediment
	TW62	105	6.23	645	0.2	5.30	1385	-Ve	-Ve	-Ve	No Sediment
TW63	120	6.16	755	0.2	5.40	1105	-Ve	-Ve	-Ve	No Sediment	
Ward-8	TW64	60	7.42	780	0.4	6.40	1165	-Ve	+Ve	-Ve	No Sediment
	TW65	60	6.27	659	0.3	5.50	1230	+Ve	-Ve	-Ve	Mild
	TW66	75	6.19	754	0.5	5.15	1835	-Ve	-Ve	-Ve	Moderate
	TW67	105	5.96	544	0.3	4.95	835	-Ve	-Ve	-Ve	No Sediment
	TW68	105	7.82	694	0.3	5.75	965	-Ve	-Ve	-Ve	No Sediment
	TW69	90	6.97	732	0.3	5.60	1125	-Ve	+Ve	-Ve	Mild
	TW70	105	7.62	525	0.2	6.10	870	-Ve	-Ve	-Ve	No Sediment
	TW71	120	7.39	514	0.3	6.80	915	-Ve	-Ve	-Ve	No Sediment
TW72	120	6.34	680	0.4	5.70	1015	-Ve	+Ve	-Ve	No Sediment	

#### Determination of microbial load:

~~Present~~ [This](#) study [also](#) showed that all of the tube-wells water samples contained a variety of Microorganisms (Table 2). Out of seventy-two tube-wells water, seventeen contained more fecal coliforms than the recommended limit set by WHO (23.61% of the sample) (Khan *et al.*, 2013). There was a significant association found between tube-well water contamination with fecal coliforms and distance of tube-well from the latrine. All the tube-well waters that contained fecal coliforms were within 30 feet from the latrine with exception of TW69, which was found distance 41 feet from the latrine. According to the results in Table 2, it was clear that the presence of fecal coliforms in the tube-well water was directly related to the surrounding latrine condition and distance from the latrine. In a similar study on analysis of tube-well water from Fulbaria pourasava in Mymensingh district of Bangladesh, it was reported that, 32% water were contaminated by fecal



coliforms of which 30% of water were contaminated with total coliforms (TC) than the recommended limits ( $\leq 10$  coliforms/100 ml water) (Islam *et al.*, 2001). There was no significant relationship between deepness of the tube well with the contamination of fecal coliforms.

Amount of heterotrophic plate count (HPC) and total coliform count (TCC) contained in the tube-well water samples of 8 different locations of Nalitabari township of Sherpur district were summarized in Table 3. It showed that all water sources (100%) contained total coliforms (TC) ranging from  $\leq 2$  cfu/100 ml to 130 cfu/100 ml and HPC ranging from  $1.0 \times 10^3$  cfu/ml up to  $7.5 \times 10^3$  cfu/ml. Twenty-six water samples were contained more TCC than the accepted limit and 76.92% of these tube-wells were located within 30 feet away from latrine. Among them TW13, TW15, TW50, TW66, and TW67 were highly polluted with TCC which has 110 cfu/100 ml, 95 cfu/100 ml, 130 cfu/100 ml, 90 cfu/100 ml and 130 cfu/100 ml of TCC, respectively whereas accepted limit in Bangladesh is only up-to 10 coliforms/100 ml water (Kabir *et al.*, 2015). Tube-well number TW13, TW15, TW50, TW66, and TW67 were only 11 feet, 6 feet, 15 feet, 26 feet and 8 feet away from the latrines, respectively. Highest TCC was found in the sample of TW67 in Ward-8 and TW50 in Ward-6 and the highest value was 130 cfu/100 ml. There was a significant association between tube-well water contamination with total coliforms or HPC and surrounding latrine condition. The highest HPC count was found in tube-well water sample of TW67, was  $7.5 \times 10^3$  cfu/ml. This tube-well was only 8 feet away from latrine. Water sample TW3, TW4, TW16, TW27, TW35, TW42, TW51 and TW61 contained very lowest amount of HPC count and that was  $1 \times 10^3$  cfu/ml. The mean HPC was observed  $1.78 \times 10^3$  cfu/ml in Ward- 1,  $2.66 \times 10^3$  cfu/ml in Ward- 2,  $2.83 \times 10^3$  cfu/ml in Ward- 3,  $2.20 \times 10^3$  cfu/ml in Ward- 4,  $2.99 \times 10^3$  cfu/ml in Ward- 5,  $2.76 \times 10^3$  cfu/ml in Ward -6,  $3.01 \times 10^3$  cfu/ml in Ward- 7 and  $3.24 \times 10^3$  cfu/ml in Ward- 8. It has been generally believed in Bangladesh that groundwater is relatively free of microorganisms and, therefore, suitable for human consumption without treatment. However, the results of this study clearly showed that all samples of tube-well water in Bangladesh that were examined contained different counts of bacteria more than permissible limit (Prosun *et al.*, 2018).

**Table 2: Microbiological analysis of tube-well water**

Sample site	Tube well water (TW)	Deepness of the Tube well (feet)	Latrine Distance (feet)	Surrounding Latrine Condition	HPC (cfu/ml)	Mean HPC (cfu/ml)	Coliform count (TCC) / (100ml)	Fecal Coliforms
Ward- 1	TW1	45	5	Direct pit	$4.5 \times 10^3$	$1.78 \times 10^3$	72	+Ve
	TW2	60	7	Offset	$1.2 \times 10^3$		14	+Ve
	TW3	45	3	Offset	$1.0 \times 10^3$		27	+Ve
	TW4	75	4	SWST	$1.0 \times 10^3$		5	-Ve
	TW5	90	12	Direct pit	$1.1 \times 10^3$		7	-Ve

	TW6	90	16	Direct pit	$2.5 \times 10^3$		11	-Ve
	TW7	120	30	Direct pit	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW8	135	21	Direct pit	$2.0 \times 10^3$		22	-Ve
	TW9	150	34	Direct pit	$1.2 \times 10^3$		$\leq 2$	-Ve
Ward- 2	TW10	45	55	Direct pit	$1.5 \times 10^3$	$2.66 \times 10^3$	23	-Ve
	TW11	45	22	Offset	$2.5 \times 10^3$		52	-Ve
	TW12	45	16	Direct pit	$1.5 \times 10^3$		12	-Ve
	TW13	105	11	Direct pit	$3.7 \times 10^3$		110	+Ve
	TW14	75	34	Direct pit	$4.2 \times 10^3$		$\leq 2$	-Ve
	TW15	90	6	Direct pit	$5.6 \times 10^3$		95	+Ve
	TW16	150	28	Direct pit	$1.0 \times 10^3$		6	-Ve
	TW17	135	47	SWST	$2.5 \times 10^3$		$\leq 2$	-Ve
	TW18	150	40	Direct pit	$1.5 \times 10^3$		$\leq 2$	-Ve
Ward- 3	TW19	60	8	Direct pit	$3.5 \times 10^3$	$2.83 \times 10^3$	5	-Ve
	TW20	60	13	Direct pit	$1.5 \times 10^3$		7	-Ve
	TW21	60	5	Direct pit	$3.7 \times 10^3$		4	+Ve
	TW22	90	56	Direct pit	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW23	90	54	SWST	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW24	105	45	Direct pit	$4.5 \times 10^3$		32	-Ve
	TW25	120	26	Offset	$1.1 \times 10^3$		$\leq 2$	-Ve
	TW26	120	28	Offset	$2.0 \times 10^3$		$\leq 2$	-Ve
	TW27	120	35	Direct pit	$1.0 \times 10^3$		$\leq 2$	-Ve
Ward- 4	TW28	60	50	Offset	$4.2 \times 10^3$	$2.20 \times 10^3$	8	-Ve
	TW29	60	24	Offset	$1.5 \times 10^4$		55	-Ve
	TW30	75	28	Direct pit	$2.0 \times 10^3$		14	-Ve
	TW31	105	38	Direct pit	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW32	90	27	Direct pit	$1.1 \times 10^3$		$\leq 2$	-Ve
	TW33	105	43	Offset	$1.5 \times 10^3$		5	-Ve
	TW34	135	18	Direct pit	$4.0 \times 10^3$		26	+Ve
	TW35	120	12	Offset	$1.0 \times 10^3$		20	-Ve
	TW36	120	62	Offset	$3.0 \times 10^3$		$\leq 2$	-Ve
Ward- 5	TW37	75	55	Offset	$4.2 \times 10^3$	$2.99 \times 10^3$	29	-Ve
	TW38	60	26	Direct pit	$1.5 \times 10^3$		26	-Ve
	TW39	75	16	Direct pit	$3.5 \times 10^3$		8	+Ve
	TW40	90	15	Direct pit	$3.0 \times 10^3$		5	+Ve
	TW41	105	34	Direct pit	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW42	90	44	Offset	$1.0 \times 10^3$		5	-Ve
	TW43	105	46	Direct pit	$2.7 \times 10^3$		$\leq 2$	-Ve
	TW44	90	32	SWST	$5.0 \times 10^3$		15	-Ve
	TW45	120	21	Direct pit	$4.5 \times 10^3$		37	+Ve
Ward- 6	TW46	75	60	Offset	$2.5 \times 10^3$	$2.76 \times 10^3$	20	-Ve
	TW47	75	30	Offset	$1.5 \times 10^3$		$\leq 2$	-Ve
	TW48	75	42	Offset	$2.0 \times 10^3$		10	-Ve
	TW49	105	21	Direct pit	$1.1 \times 10^3$		5	-Ve
	TW50	90	15	Direct pit	$7.0 \times 10^3$		130	+Ve

	TW51	90	10	Direct pit	$1.0 \times 10^3$		7	-Ve
	TW52	105	34	Direct pit	$3.5 \times 10^3$		∞	-Ve
	TW53	90	45	Offset	$2.5 \times 10^3$		∞	-Ve
	TW54	120	36	Direct pit	$3.7 \times 10^3$		∞	-Ve
Ward- 7	TW55	45	18	Direct pit	$5.5 \times 10^3$	$3.01 \times 10^3$	67	-Ve
	TW56	75	15	Offset	$1.5 \times 10^3$		∞	+Ve
	TW57	75	43	Offset	$1.1 \times 10^3$		∞	-Ve
	TW58	90	19	SWST	$2.0 \times 10^3$		∞	+Ve
	TW59	90	15	Direct pit	$3.5 \times 10^3$		43	-Ve
	TW60	105	23	Direct pit	$2.5 \times 10^3$		9	-Ve
	TW61	90	29	Direct pit	$1.0 \times 10^3$		∞	-Ve
	TW62	105	13	Direct pit	$4.5 \times 10^3$		24	-Ve
Ward- 8	TW63	120	9	Direct pit	$5.5 \times 10^3$	$3.24 \times 10^3$	∞	+Ve
	TW64	60	17	Offset	$1.5 \times 10^3$		∞	+Ve
	TW65	60	45	Offset	$3.5 \times 10^3$		5	-Ve
	TW66	75	26	Direct pit	$5.5 \times 10^3$		90	-Ve
	TW67	105	8	Direct pit	$7.5 \times 10^3$		130	-Ve
	TW68	105	39	Direct pit	$1.0 \times 10^3$		16	-Ve
	TW69	90	41	Direct pit	$2.5 \times 10^3$		9	+Ve
	TW70	105	25	Offset	$1.5 \times 10^3$		∞	+Ve
TW71	120	51	Direct pit	$2.0 \times 10^3$	∞	-Ve		
TW72	120	34	Direct pit	$4.2 \times 10^3$	5	-Ve		

SWST: Soak well with septic tank, HPC: Heterotrophic plate count

### Biochemical test for bacterial analysis

Among the isolates, two kinds of bacteria (*E. coli* and *Vibrio cholerae*) were confirmed based on biochemical experiments. The results of biochemical tests for isolates from water samples were summarized in Table 3.

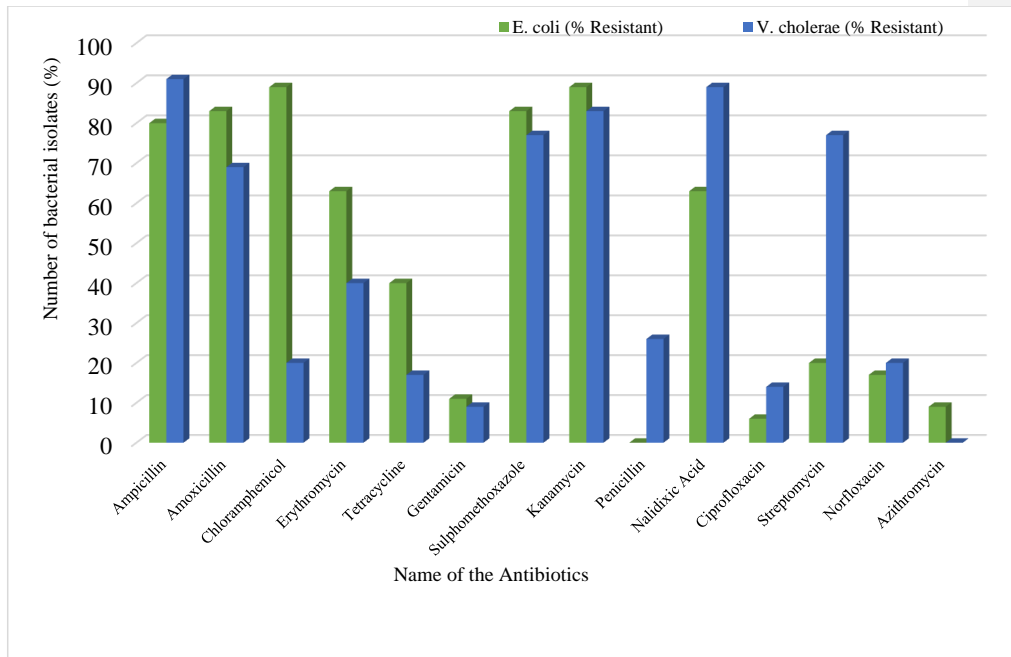
**Table 3: Biochemical analysis of the isolated bacteria**

Gram Staining	Biochemical reaction															Presumptive Bacteria	
	EMB plate	KIA			MIU			Simon' s Citrate	VP test	Oxidase	Catalase	Mannitol	Starch hydrolysis	Methyl Red	Glucose		Lactose fermentation test
	Slant	Bud	Gas	Motility	Indole	Urease											
-Ve	+	A	A	+	+	+	+	-	-	-	+	A	-	+	A G	+	<i>E. coli</i>

-Ve	-	A	A	-	+	+	-	+	-	+	+	+	-	+	-	-	<i>Vibrio cholerae</i>
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**Antibiotic susceptibility test**

Nowadays antibiotic-resistant bacteria [is](#) a great threat to our health and environment as well as acts as a culprit in medical health care. These bacteria might have gained resistance property due to the indiscriminate use of antibiotics. -In this study 20 *Vibrio cholerae* and 30 *E. coli* bacteria were taken under antibiogram experiment. These antibiogram experiments revealed that *Vibrio cholerae* was resistant to Ampicillin (91%), Nalidixic Acid (89%), Kanamycin (83%), and Amoxicillin (69%). Mobile genetic elements are responsible for the spreading of drug resistance genes in *V. cholerae* strains in response to quorum sensing signaling. Thus tetracycline-resistant El Tor strains of *V. cholerae* was re-emerged in Bangladesh in 1991 (Fazil and Singh, 2011). On the other hand, *E. coli* showed higher resistance to Chloramphenicol (89%), Kanamycin (89%), Amoxicillin (83%), and Sulphomethoxazole (83%) (Figure-1). Other studies also showed that *E. coli* isolates were established antibiotic resistance upon commercially used antibiotic like as Streptomycin, Sulfamethoxazole, Tetracycline, Ampicillin, and so on (Singh *et al.*, 2005; Tadesse *et al.*, 2012).



**Figure 1:** Antibigram profiles of *E. coli* and *V. cholerae*

**Diseases-Health impact related to bacterial contamination and heavy metal toxicity:**

Survey [report in the current study area analysis](#) showed that some adults and children were affected by different diseases related to microbial infection (Table 4). Out of 400 individuals, 1.75% were suffering from diarrhea and 0.5% were suffering from dysentery from a long time (Table 4). Children were more frequently affected by diarrhea and prone to these diseases than adult. According to some study, children under the age of five are the more susceptible group accounting for major part in deaths due to diarrhea or diarrheal diseases (Thiam *et al.*, 2017; Black, Morris and Bryce, 2003). Among 200 adults, 6.5% individuals were rarely affected by typhoid where 1% adults were affected within last month. 6% adult and 4.5% children were rare or sometime affected by Salmonellosis where within last month 2.5% adult and 0.5% child were affected. No respondents were affected by Campylo-bacteriosis found in this study (Table 4).

**Table 4: Diseases related to microbial contamination in drinking water**

Diseases	Suffering	Child (Total-200)			Adult (Total-200)		
		Frequently	Rare or sometime	Affected	Frequently	Rare or sometime	Affected

	From a long time			within last month			within last month
Diarrhea	7	11	8	13	2	4	11
Dysentery	2	3	6	1	-	7	6
Cholera	-	2	8	3	3	5	1
Typhoid	-	-	3	1	-	13	2
Hepatitis	-	-	3	1	-	9	2
Botulism	-	-	4	-	-	6	3
Campylobacteriosis	-	-	-	-	-	-	-
Salmonellosis	-	2	9	1	7	12	5

Some respondents were also affected by diseases related to heavy metal toxicity such as scabies skin diseases, neurological problems, bad headache and anemia (Table 5). About 3.25% and 4.5% respondents were suffering from a ~~long-time~~ long-time skin diseases and bad headache, respectively. Adult were more frequently affected by skin diseases and bad headache than children, only 1% and 1.5% individual children out of 200 children were frequently affected by skin diseases and bad headache, respectively. On the other hand, out of 200 adults 8.5% and 3% were frequently affected by skin diseases and bad headache, respectively. No respondents were reported in this study to affect by lead poisoning and arsenicosis because the water of the study area ~~were~~was not contaminated by lead and rarely contaminated by arsenic (6.94%).

**Table 5: Diseases related to heavy metal toxicity**

Diseases	Suffering From a long time	Child (Total-200)			Adult (Total-200)		
		Frequently	Rare or sometime	Affected within last month	Frequently	Rare or sometime	Affected within last month
Lead poisoning	-	-	-	-	-	-	-
Arsenicosis	-	-	-	-	-	-	-
Scabies	2	-	-	-	3	1	1
Skin diseases	13	2	3	3	17	22	6
Skin cancer	-	-	-	-	-	-	-
Liver cirrosis	-	-	-	-	-	-	-
Neurological problems	3	-	-	-	-	-	-
Bad headache	18	3	8	12	6	15	19
Kidney damage	-	-	-	-	-	-	-
Anemia	1	-	-	-	-	-	-
Multiple sclerosis	-	-	-	-	-	-	-

Muscular dystrophy	-	-	-	-	-	-	-
Parkinson's disease	-	-	-	-	-	-	-
Alzheimer's disease	-	-	-	-	-	-	-
Miscarriage	-	-	-	-	-	2	-
Lung diseases	5	-	-	-	-	3	-

### Conclusion

In this study, it was found that the tube-well which was close to latrine was more susceptible to contamination with fecal coliform. If the surrounding area was more polluted, then there was more chance of contamination. Heterotrophic plate count (HPC) was high in some tube-well water which may be due to polluted earth environment. Identification of *E. coli* and *Vibrio cholerae* in the tube-well water indicated poor sanitation condition in the study area. Maximum tube-well water samples were negative to arsenic, only a few about 6.94% had arsenic pollution. A proper sanitation and drainage network system in the township must get a priority. All tube-well should be far away from polluted earth environment and distance of tube-well from latrine should be minimum 40-50 feet. ~~The conclusion of the study is that~~ The tube-well water of the studied area in Bangladesh cannot be considered safe for drinking unless properly treatment. For developing a modern township drinking water must be free from hazards which are threaten to public health.

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**Commented [S10]:** Try to use more recent references from good journals in the introduction, and results and discussion section, and revise this section according to it.

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