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Appraisal of Heavy Metal Presence and Water Quality having Microbial Load and Associated Human Health Risk: A study on tube-well water in Nalitabari township of Sherpur district, Bangladesh

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Abstract

This article is based on a study aimed to determine physiochemical parameters, fecal coliform, total coliforms, heterotrophic plate count, arsenic, iron and lead of water to evaluate their effects on human health. Analysis was carried out on tube-well water collected from Nalitabari township of Sherpur District in Bangladesh. The dissolved oxygen (DO), total dissolved solids (TDS), salinity and electrical conductivity were in the ranges of 4.30 to 7.30 ppm, 350 to 792 mg/l, 0.2 to 0.5%, and 715 to 1,970 µS/cm. The pH values were slightly lesser or more than permissible value. Due to the vicinity to the latrines, 17 tube-well water was contaminated by fecal coliforms. The highest heterotrophic plate count was 7.5×10^3 cfu/ml in ward-8 of the town. *Eschericia coli* and *Vibrio cholerae* were identified in ratio of 30.56% and 18.06%, respectively, in the tube-well water, resulting into diarrhea among children. About 6.94% of tube-well water was contaminated with arsenic. 3.25% and 4.5% respondents were suffering from skin diseases and headache, respectively. So, an alternative source of drinking water should be arranged for a better public health of present and next generations.

Keywords

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 and irrigation system in Bangladesh. The shallow alluvial aquifers receive water through rainfall and flooding. The static water level in much of Bangladesh is due to its availability within 7 meter of the ground surface round the year. 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 such as drinking and cooking purposes. It is a matter of great concern that the drinking water is getting polluted with various organic and inorganic matters (Rezania *et al*., 2015). Depending on the 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 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 protozoans that can be introduced by anthropogenic activities and poor sanitation. According to World Health Organization, 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 prevents and controls diarrheal diseases (Motarjemi and Käferstein, 1999; Yager *et al*., 2006). Drinking water quality among the natural parameters, such 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 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. From about 7-11 million hand pumped tube-wells, approximately half of them have been estimated supplying groundwater with an arsenic concentration more than 50 micrograms/l, which is the maximum level of arsenic allowed in a drinking water (Rahman *et al*., 2018; 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). It was estimated that about 80% of the diseases in developing countries are attributed 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 sanitation facilities in terms of quality and quantity are utmost necessities for assessing the living condition of the urban and semi-urban areas of Bangladesh. Due to poor sanitation and unawareness about personal hygiene practices, drinking water is contaminated by some pathogenic bacteria and increases the risks of water-borne diseases (Suthar, Chhimpa and Singh, 2009).

Besides, the presence of heavy metals in drinking water is a matter of great concern due to their impacts on human life. Contamination of tube-well water with arsenic and heavy metal is hazardous for health. People are suffering from headaches, abdominal pain, cancer, kidney damage, nerve damages and skeletal damages due to the toxic effects of these metals (Rasool *et al*., 2016). Therefore, the present study was designed to evaluate tube-well water quality and to identify the presence of heavy metal contaminations in tube-well water. The objectives of this study were also to investigate the tube-well water's physiochemical parameters (such as dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS) and pH), microbial load of tube-well water and their health impact on people of Nalitabari Township of Sherpur District in Bangladesh.

Materials and Methods

Study Area

Nalitabari is an Upazila (sub-district) of Sherpur District under the Division of Mymensingh in Bangladesh. It is located between 25°01' and 25°13' N latitudes and between 90°04' and 90°19' E longitudes. It is 174.9 kilometer away from Dhaka and situated on the bank of the river Bhogai in northern part of Bangladesh. Nalitabari municipality is one of the oldest municipalities in Bangladesh, established on 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 immediately transported to the laboratory 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 (Reddy *et al*., 2020).

Heterotrophic Plate Count (HPC)

For determination of heterotrophic plate count, 100 micro liters 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 37ºC 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 form 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 tests (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; Ali *et al*., 2020).

Assessment of Health Impact

A semi-structured questionnaire was prepared for field investigation to evaluate the health impact of the people who used these tube-well waters. Total 400 (200 children and 200 adults, among them 50% were male and 50% were female) respondents of the study area were interviewed to determine the health status of people in the study area (Rakib *et al.,* 2019; Ali *et al*. 2020).

Results and Discussions

Physiochemical Properties of Water

Amounts 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 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 was acidic and lower than recommended by the World Health Organization (Robinson *et al*., 2011).

The DO, TDS, salinity, and conductivity of water samples were in the ranges of 4.30 to 7.30 ppm, 350 to 792 mg/l, 0.2 to 0.5%, and 715 to 1970 µS/cm, respectively. The mean DO content of all water samples was 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 was found 3.95 mg/l in TW15 belonging to ward-2. The value of DO of all water samples was not satisfactory, as the standard value is 6.00 mg/l or more for 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 than1200 mg/liter (Beyene, 2015). So, all the values of TDS were in an acceptable range. The result of the study showed that the electrical conductivity (EC) of 50% water samples was within the standard value of drinking water in Bangladesh. The maximum permissible limit of EC in Bangladesh is 1,200 µS/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 µS/cm and was well within the permissible limit of 400 µS/cm as set by WHO guideline (Asif *et al*., 2015). All the tube-wells water of the study area was within the acceptable salinity range where salinity of the freshwater is 0 to 0.5%. There was a significant relationship among the salinity, TDS and EC. In this study, it was found when the salinity of tube-wells water was 0.5% or more, the TDS and EC values were also high. Another study suggested that most of the physicochemical parameters of groundwater in Rajshahi city were not at the alarming stage (Rasul and Jahan, 2010).

Presence of Heavy Metals

This study revealed that there was 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 depth within 90 feet. Out of 72 tube-wells water, 16 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, while 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 samples contain moderate sediment and 76% samples contain no sediment after centrifuge at 10,000 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 contained naturally occurring arsenic with concentration levels often significantly exceeding 10 µg/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 a water with arsenic concentrations more than 50 µg/l and 200µg/l (George *et al*., 2012), respectively. 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 50µg/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).

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 mg/l*	*EC µS/cm*	*Arsenic (As)*	*Iron (Fe)*	*Lead (Pb)*	*Sediment (After centrifuge at 10,000 rpm)*
Ward-1	TW1	45	6.58	544	0.50	4.30	1,460	-Ve	-Ve	-Ve	Moderate
	TW2	60	7.12	792	0.30	7.10	1,280	-Ve	+Ve	-Ve	Mild
	TW3	45	7.23	632	0.30	6.25	1,135	-Ve	+Ve	-Ve	Moderate
	TW4	75	7.74	556	0.20	5.50	920	-Ve	-Ve	-Ve	No Sediment
	TW5	90	7.19	782	0.20	6.25	975	+Ve	-Ve	-Ve	No Sediment
	TW6	90	6.42	624	0.30	6.65	1,070	-Ve	+Ve	-Ve	No Sediment
	TW7	120	6.78	412	0.20	5.55	860	-Ve	-Ve	-Ve	No Sediment
	TW8	135	6.41	669	0.40	5.15	1,135	-Ve	-Ve	-Ve	No Sediment
	TW9	150	6.96	424	0.20	4.95	785	-Ve	-Ve	-Ve	No Sediment
Ward- 2	TW10	45	6.97	534	0.20	5.10	935	-Ve	-Ve	-Ve	Mild
	TW11	45	6.69	433	0.50	4.95	1,545	-Ve	+Ve	-Ve	Moderate
	TW12	45	7.05	355	0.50	6.20	1,960	+Ve	+Ve	-Ve	Moderate
	TW13	105	6.38	475	0.20	4.85	795	-Ve	-Ve	-Ve	No Sediment
	TW14	75	6.22	676	0.30	4.15	1,320	-Ve	-Ve	-Ve	No Sediment
	TW15	90	6.12	765	0.30	3.95	1,290	-Ve	-Ve	-Ve	No Sediment
	TW16	150	7.33	690	0.20	7.15	1,035	-Ve	-Ve	-Ve	No Sediment
	TW17	135	6.55	434	0.20	5.35	885	-Ve	-Ve	-Ve	No Sediment
	TW18	150	7.17	482	0.20	6.45	765	-Ve	-Ve	-Ve	No Sediment
Ward- 3	TW19	60	6.42	593	0.20	5.20	925	-Ve	-Ve	-Ve	No Sediment
	TW20	60	6.98	648	0.30	6.85	1,260	-Ve	-Ve	-Ve	No Sediment
	TW21	60	6.25	456	0.40	5.05	1,765	-Ve	-Ve	-Ve	Moderate
	TW22	90	7.62	350	0.20	7.30	825	-Ve	-Ve	-Ve	No Sediment
	TW23	90	7.17	488	0.20	6.45	785	-Ve	-Ve	-Ve	No Sediment
	TW24	105	6.02	396	0.20	4.90	765	-Ve	-Ve	-Ve	No Sediment
	TW25	120	7.92	534	0.40	6.75	1,135	-Ve	-Ve	-Ve	No Sediment
	TW26	120	6.63	452	0.20	6.40	795	-Ve	-Ve	-Ve	No Sediment
	TW27	120	7.82	745	0.30	5.85	1,530	-Ve	-Ve	-Ve	No Sediment
Ward- 4	TW28	60	6.32	675	0.40	4.45	1,745	-Ve	-Ve	-Ve	Mild
	TW29	60	6.83	780	0.30	6.90	1,340	-Ve	+Ve	-Ve	No Sediment
	TW30	75	6.64	457	0.50	6.35	1,925	+Ve	-Ve	-Ve	Moderate
	TW31	105	7.15	455	0.20	5.45	950	-Ve	-Ve	-Ve	No Sediment
	TW32	90	7.31	590	0.30	6.55	1,105	-Ve	+Ve	-Ve	No Sediment
	TW33	105	6.88	358	0.20	5.05	715	-Ve	-Ve	-Ve	No Sediment
	TW34	135	6.29	448	0.20	4.85	810	-Ve	-Ve	-Ve	No Sediment
	TW35	120	7.69	725	0.30	6.65	1,375	-Ve	-Ve	-Ve	No Sediment
	TW36	120	6.37	675	0.30	5.70	1,550	-Ve	-Ve	-Ve	No Sediment
Ward- 5	TW37	75	6.30	695	0.20	5.15	1,345	-Ve	-Ve	-Ve	No Sediment
	TW38	60	7.07	455	0.40	6.45	1,730	-Ve	-Ve	-Ve	Moderate
	TW39	75	6.32	670	0.30	5.40	1,225	-Ve	-Ve	-Ve	No Sediment
	TW40	90	6.43	575	0.40	5.70	1,345	-Ve	-Ve	-Ve	Mild

Sample site	*Tube-well water (TW)*	*Deepness of the Tube-well (feet)*	*pH*	*TDS mg/l*	*Salinity (%)*	*DO mg/l*	*EC µS/cm*	*Arsenic (As)*	*Iron (Fe)*	*Lead (Pb)*	*Sediment (After centrifuge at 10,000 rpm)*
	TW41	105	7.36	552	0.30	7.05	990	-Ve	-Ve	-Ve	No Sediment
	TW42	90	7.27	764	0.20	6.50	1,280	-Ve	-Ve	-Ve	No Sediment
	TW43	105	6.53	572	0.20	5.40	1,025	-Ve	-Ve	-Ve	No Sediment
	TW44	90	5.98	763	0.40	4.85	1,320	-Ve	+Ve	-Ve	No Sediment
	TW45	120	6.18	726	0.30	5.30	1,165	-Ve	-Ve	-Ve	No Sediment
Ward- 6	TW46	75	6.64	385	0.40	6.55	1,905	+Ve	+Ve	-Ve	No Sediment
	TW47	75	7.19	732	0.30	6.15	1,455	-Ve	+Ve	-Ve	No Sediment
	TW48	75	6.78	567	0.30	7.10	1,290	-Ve	+Ve	-Ve	Mild
	TW49	105	7.45	678	0.30	5.95	955	-Ve	-Ve	-Ve	No Sediment
	TW50	90	5.88	497	0.20	4.55	920	-Ve	-Ve	-Ve	No Sediment
	TW51	90	6.84	675	0.20	5.70	1,295	-Ve	+Ve	-Ve	No Sediment
	TW52	105	6.39	725	0.40	6.05	1,365	-Ve	-Ve	-Ve	No Sediment
	TW53	90	6.73	467	0.20	6.60	970	-Ve	-Ve	-Ve	No Sediment
	TW54	120	6.55	785	0.30	5.35	1,390	-Ve	-Ve	-Ve	No Sediment
Ward- 7	TW55	45	6.05	773	0.50	5.60	1,970	-Ve	-Ve	-Ve	Moderate
	TW56	75	6.94	780	0.20	6.40	1,325	-Ve	-Ve	-Ve	Mild
	TW57	75	7.58	743	0.30	5.05	1,195	-Ve	+Ve	-Ve	Mild
	TW58	90	6.89	743	0.20	6.25	1,080	-Ve	-Ve	-Ve	No Sediment
	TW59	90	6.36	674	0.30	5.20	1,190	-Ve	-Ve	-Ve	No Sediment
	TW60	105	6.65	533	0.20	7.10	885	-Ve	-Ve	-Ve	No Sediment
	TW61	90	7.45	696	0.20	5.75	1,365	-Ve	-Ve	-Ve	No Sediment
	TW62	105	6.23	645	0.20	5.30	1,385	-Ve	-Ve	-Ve	No Sediment
	TW63	120	6.16	755	0.20	5.40	1,105	-Ve	-Ve	-Ve	No Sediment
Ward- 8	TW64	60	7.42	780	0.40	6.40	1,165	-Ve	+Ve	-Ve	No Sediment
	TW65	60	6.27	659	0.30	5.50	1,230	+Ve	-Ve	-Ve	Mild
	TW66	75	6.19	754	0.50	5.15	1,835	-Ve	-Ve	-Ve	Moderate
	TW67	105	5.96	544	0.30	4.95	835	-Ve	-Ve	-Ve	No Sediment
	TW68	105	7.82	694	0.30	5.75	965	-Ve	-Ve	-Ve	No Sediment
	TW69	90	6.97	732	0.30	5.60	1,125	-Ve	+Ve	-Ve	Mild
	TW70	105	7.62	525	0.20	6.10	870	-Ve	-Ve	-Ve	No Sediment
	TW71	120	7.39	514	0.30	6.80	915	-Ve	-Ve	-Ve	No Sediment
	TW72	120	6.34	680	0.40	5.70	1,015	-Ve	+Ve	-Ve	No Sediment

Determination of Microbial Load

This study also showed that all the tube-well water samples contained a variety of microorganisms (Table 2). Out of 72 tube-wells, water of 17 contained more fecal coliforms than the recommended limit set by WHO (23.61% of the samples) (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 at a distance of 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 samples were contaminated by fecal coliforms of which 30% of samples were contaminated with total coliforms (TC) than the recommended limits (≤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 ≤2 cfu/100 ml to 130 cfu/100 ml and HPC ranging from 1.0×10^3 cfu/ ml up to 7.5×10^3 cfu/ ml. Twenty-six water samples contained more TCC than the permissible 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 have 110 cfu/100 ml, 95 cfu/100 ml, 130 cfu/100 ml, 90 cfu/100 ml and 130 cfu/100 ml of TCC, respectively, against permissible limit in Bangladesh of 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, which was 7.5×10^3cfu/ml. This tube-well was only 8 feet away from latrine. Water samples TW3, TW4, TW16, TW27, TW35, TW42, TW51 and TW61 contained very lowest amount of HPC count and that was 1×10^3cfu/ml in each of the samples. The mean HPC was observed 1.78×10^3 cfu/ml in ward-1, 2.66×10^3cfu/ml in ward-2, 2.83×10^3cfu/ml in ward-3, 2.20×10^3cfu/ml in ward-4, 2.99×10^3cfu/ml in ward-5, 2.76×10^3cfu/ml in ward-6, 3.01×10^3cfu/ml in ward-7 and 3.24×10^3 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, which are above permissible limit (Prosun *et al*., 2018).

Table 2: Microbiological analysis of tube-well water

Sample site	*Tube-well water (TW)*	*Depth of the Tube-well (feet)*	*Latrine Distance (feet)*	*Surrounding Latrine Condition*	*HPC (cfu/ml)*	*Mean HPC (cfu/ml)*	*Coli-form count (TCC) / (100ml)*	*Fecal Coli-forms*
Ward- 1	TW1	45	5	Direct pit	4.5×10^3	1.78×10^3	72	+Ve
	TW2	60	7	Offset	1.2×10^3		14	+Ve
	TW3	45	3	Offset	1.0×10^3		27	+Ve
	TW4	75	4	SWST	1.0×10^3		5	-Ve
	TW5	90	12	Direct pit	1.1×10^3		7	-Ve
	TW6	90	16	Direct pit	2.5×10^3		11	-Ve
	TW7	120	30	Direct pit	1.5×10^3		≤2	-Ve
	TW8	135	21	Direct pit	2.0×10^3		22	-Ve
	TW9	150	34	Direct pit	1.2×10^3		≤2	-Ve
Ward- 2	TW10	45	55	Direct pit	1.5×10^3	2.66×10^3	23	-Ve
	TW11	45	22	Offset	2.5×10^3		52	-Ve
	TW12	45	16	Direct pit	1.5×10^3		12	-Ve
	TW13	105	11	Direct pit	3.7×10^3		110	+Ve
	TW14	75	34	Direct pit	4.2×10^3		≤2	-Ve
	TW15	90	6	Direct pit	5.6×10^3		95	+Ve
	TW16	150	28	Direct pit	1.0×10^3		6	-Ve
	TW17	135	47	SWST	2.5×10^3		≤2	-Ve
	TW18	150	40	Direct pit	1.5×10^3		≤2	-Ve
Ward- 3	TW19	60	8	Direct pit	3.5×10^3	2.83×10^3	5	-Ve
	TW20	60	13	Direct pit	1.5×10^3		7	-Ve
	TW21	60	5	Direct pit	3.7×10^3		4	+Ve
	TW22	90	56	Direct pit	1.5×10^3		≤2	-Ve
	TW23	90	54	SWST	1.5×10^3		≤2	-Ve
	TW24	105	45	Direct pit	4.5×10^3		32	-Ve
	TW25	120	26	Offset	1.1×10^3		≤2	-Ve

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Sample site	*Tube-well water (TW)*	*Depth of the Tube-well (feet)*	*Latrine Distance (feet)*	*Surrounding Latrine Condition*	*HPC (cfu/ml)*	*Mean HPC (cfu/ml)*	*Coli-form count (TCC) / (100ml)*	*Fecal Coli-forms*
	TW26	120	28	Offset	2.0×10^3		≤2	-Ve
	TW27	120	35	Direct pit	1.0×10^3		≤2	-Ve
Ward- 4	TW28	60	50	Offset	4.2×10^3	2.20×10^3	8	-Ve
	TW29	60	24	Offset	1.5×10^4		55	-Ve
	TW30	75	28	Direct pit	2.0×10^3		14	-Ve
	TW31	105	38	Direct pit	1.5×10^3		≤2	-Ve
	TW32	90	27	Direct pit	1.1×10^3		≤2	-Ve
	TW33	105	43	Offset	1.5×10^3		5	-Ve
	TW34	135	18	Direct pit	4.0×10^3		26	+Ve
	TW35	120	12	Offset	1.0×10^3		20	-Ve
	TW36	120	62	Offset	3.0×10^3		≤2	-Ve
Ward- 5	TW37	75	55	Offset	4.2×10^3	2.99×10^3	29	-Ve
	TW38	60	26	Direct pit	1.5×10^3		26	-Ve
	TW39	75	16	Direct pit	3.5×10^3		8	+Ve
	TW40	90	15	Direct pit	3.0×10^3		5	+Ve
	TW41	105	34	Direct pit	1.5×10^3		≤2	-Ve
	TW42	90	44	Offset	1.0×10^3		5	-Ve
	TW43	105	46	Direct pit	2.7×10^3		≤2	-Ve
	TW44	90	32	SWST	5.0×10^3		15	-Ve
	TW45	120	21	Direct pit	4.5×10^3		37	+Ve
Ward -6	TW46	75	60	Offset	2.5×10^3	2.76×10^3	20	-Ve
	TW47	75	30	Offset	1.5×10^3		≤2	-Ve
	TW48	75	42	Offset	2.0×10^3		10	-Ve
	TW49	105	21	Direct pit	1.1×10^3		5	-Ve
	TW50	90	15	Direct pit	7.0×10^3		130	+Ve
	TW51	90	10	Direct pit	1.0×10^3		7	-Ve
	TW52	105	34	Direct pit	3.5×10^3		≤2	-Ve
	TW53	90	45	Offset	2.5×10^3		≤2	-Ve
	TW54	120	36	Direct pit	3.7×10^3		≤2	-Ve
Ward- 7	TW55	45	18	Direct pit	5.5×10^3	3.01×10^3	67	-Ve
	TW56	75	15	Offset	1.5×10^3		≤2	+Ve
	TW57	75	43	Offset	1.1×10^3		≤2	-Ve
	TW58	90	19	SWST	2.0×10^3		≤2	+Ve
	TW59	90	15	Direct pit	3.5×10^3		43	-Ve
	TW60	105	23	Direct pit	2.5×10^3		9	-Ve
	TW61	90	29	Direct pit	1.0×10^3		≤2	-Ve
	TW62	105	13	Direct pit	4.5×10^3		24	-Ve
	TW63	120	9	Direct pit	5.5×10^3		≤2	+Ve
Ward- 8	TW64	60	17	Offset	1.5×10^3	3.24×10^3	≤2	+Ve
	TW65	60	45	Offset	3.5×10^3		5	-Ve
	TW66	75	26	Direct pit	5.5×10^3		90	-Ve
	TW67	105	8	Direct pit	7.5×10^3		130	-Ve
	TW68	105	39	Direct pit	1.0×10^3		16	-Ve
	TW69	90	41	Direct pit	2.5×10^3		9	+Ve
	TW70	105	25	Offset	1.5×10^3		≤2	+Ve

Sample site	*Tube-well water (TW)*	*Depth of the Tube-well (feet)*	*Latrine Distance (feet)*	*Surrounding Latrine Condition*	*HPC (cfu/ml)*	*Mean HPC (cfu/ml)*	*Coli-form count (TCC) / (100ml)*	*Fecal Coli-forms*
	TW71	120	51	Direct pit	2.0×10^3		≤2	-Ve
	TW72	120	34	Direct pit	4.2×10^3		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*
		KIA			*MIU*												
	EMB plate	*Slunt*	*Bud*	*Gas*	*Motility*	*Indole*	*Urease*	*Simon's Citrate*	*VP test*	*Oxidase*	*Catalase*	*Mannitol*	*Starch hydrolysis*	*Methyl Red*	*Glucose*	*Lactose fermentation test*	
-Ve	+	A	A	+	+	+	+	–	–	–	+	A	–	+	AG	+	*E. coli*
-Ve	-	A	A	-	+	+	-	+	-	+	+	+	-	+	-	-	*Vibrio cholerae*

Antibiotic Susceptibility Test

Nowadays antibiotic-resistant bacteria are a great threat to our health and environment as well as act 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 strains of *V. cholerae* 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).

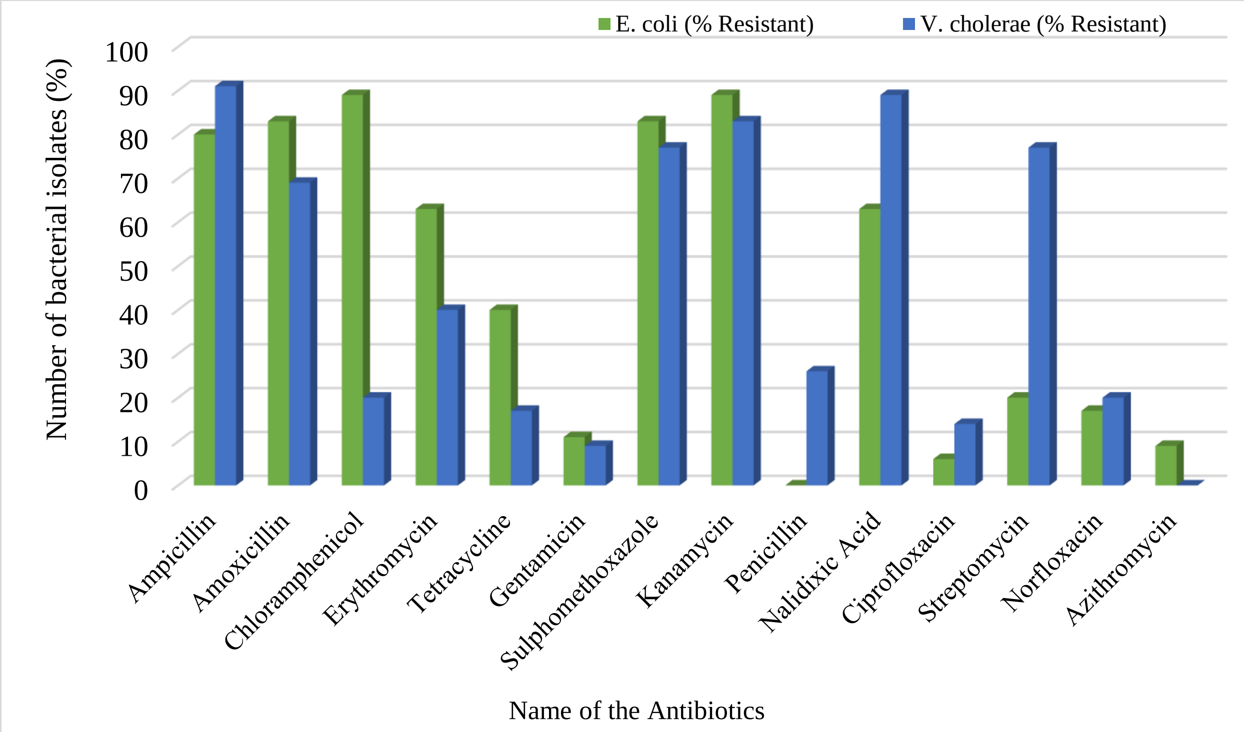

Figure 1: Antibiogram profiles of *E. coli* and *V. cholerae*

Health Impact of Bacterial Contamination and Heavy Metal Toxicity

Survey analysis showed that some adults and children were affected by different diseases attributed to microbial infection (Table 4). Out of 400 individuals, 1.75% were suffering from diarrhea and 0.5% were suffering from dysentery for a long time (Table 4). Children were more frequently affected by diarrhea and were prone to these diseases than adults. According to some other 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 whereas 1% adults were affected within last month. 6% adults and 4.5% children were rarely or sometime affected by Salmonellosis whereas within last month 2.5% adults and 0.5% children were affected. No respondents were found in this study affected by Campylobacteriosis (Table 4).

Table 4: Diseases related to microbial contamination in drinking water

Diseases	*Suffering for 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*
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
Campylo-bacteriosis	-	-	-	-	-	-	-
Salmonellosis	-	2	9	1	7	12	5

Some respondents were also affected by diseases attributed 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 skin diseases and bad headache, respectively. Adults 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 be affected by lead poisoning and arsenicosis because the water of the study area was not contaminated by lead and rarely contaminated by arsenic (6.94%).

Table 5: Diseases related to heavy metal toxicity

Diseases	Suffering for 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 cirrhosis	-	-	-	-	-	-	-
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-wells, which were close to latrine, were more susceptible to contamination with fecal coliform. When 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 in municipal functioning. All tube-wells should be far away from polluted earth environment and distance of tube-well from latrine should be minimum 40-50 feet. The tube-well water of the studied area in Bangladesh cannot be considered safe for drinking unless properly treated. For developing a modern township, drinking water must be free from hazards which are threatening the public health.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	Author 1	Author 2	Author 3	Author 4	Author 5	Author 6	Author 7
Conceived and designed the research or analysis	Yes	No	No	No	No	Yes	Yes
Collected the data and lab experiment	Yes	Yes	Yes	Yes	Yes	No	No
Contributed to data analysis & interpretation	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Wrote the article/paper	Yes	No	No	No	Yes	Yes	No
Critical revision of the article/paper	No	No	No	No	No	Yes	Yes
Editing of the article/paper	No	No	No	No	No	Yes	Yes
Supervision	No	No	No	No	No	Yes	No
Project Administration	No	No	No	No	No	No	Yes
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